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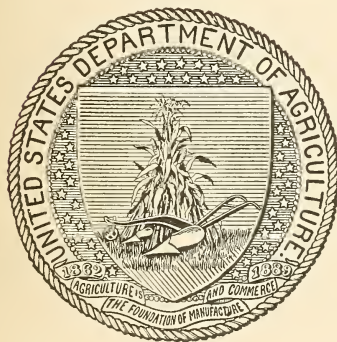
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U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF SOILS—BULLETIN No. 53.
MILTON WHITNEY, Chief.

THE ISOLATION OF HARMFUL ORGANIC
SUBSTANCES FROM SOILS.

BY

OSWALD SCHREINER AND EDMUND C. SHOREY.



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LETTER OF TRANSMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF SOILS,
Washington, D. C., May 14, 1908.

SIR: I have the honor to transmit herewith the manuscript of a technical paper entitled, The Isolation of Harmful Organic Substances from Soils, by Oswald Schreiner and Edmund C. Shorey, of this Bureau. This article is the result of a study of several unproductive soils, and deals with the properties of crystalline bodies isolated therefrom.

The material throws considerable additional light upon the question of soil fertility. The manuscript has been gone over carefully with Assistant Secretary Hays, who concurs in my recommendation for its publication as Bulletin No. 53 of the Bureau of Soils.

Respectfully,

MILTON WHITNEY,
Chief of Bureau.

Hon. JAMES WILSON,
Secretary of Agriculture.

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THE ISOLATION OF HARMFUL ORGANIC SUBSTANCES FROM SOILS.

THE PRESENCE OF HARMFUL SUBSTANCES.

Investigations into the causes of the infertility of soils have established three facts which may be considered fundamental in any discussion of the subject. These are: First, soils may be infertile because of the presence in them of some substance or substances inimical to plant growth. Second, many plants produce and possibly excrete, as the result of growth, organic compounds which are poisonous to the plants producing them. Third, many of the organic constituents of plants which on the death and decay of vegetation find their way into the soil, or compounds which may arise during subsequent changes in the soil, inhibit growth when presented in solution to the roots of growing plants.

The facts on which these three generalizations are based have been published in detail^a and will be but briefly presented here.

The essential facts regarding the first proposition are these: Aqueous extracts of soil retain the fertile or infertile properties of the soils from which they are made. A water extract of a poor soil is a poorer medium for the growth of plants than an extract of a good soil. The growth of seedlings in an extract of an infertile soil is often less than in pure distilled water. Since, however, the distilled water contains no mineral nutrients whatever, while the poor soil extract contains some, the diminished growth in the latter must be due to the presence of something harmful, something that hinders the plant's development, even in the presence of the nutrients in the soil solution and those stored in the seed. This simple experiment is sufficient to establish this, but there are a number of other observations thoroughly in accord with the conclusion stated. Dilution of a poor soil extract often makes it a better medium for the growth of seedlings than the original extract. Treatment of such an extract

^a Buls. Nos. 23, 28, 36, 40, 47, Bureau of Soils, U. S. Dept. Agr.

with some absorbing agent, such as carbon black or ferric hydrate, which furnishes no mineral plant food, has the same effect. In other words, any treatment which dilutes the inhibitory material in the soil extract makes it better even when the mineral plant food is diminished at the same time. In further accord with these observations is the fact that the addition of certain chemicals which furnish no plant food often improves poor soil extracts. Pyrogallol is such a compound, the improvement being evidently due to some change in the harmful material brought about by the added compound, or else it enables the plant to overcome the harmful effect.

The second proposition, viz, that roots may excrete harmful bodies, is based, first, on the behavior of the roots of wheat seedlings when grown in agar-agar in which wheat had been previously grown,^a and, further, on the fact that from soils in which wheat and cowpeas had been grown until the growth was greatly diminished crystalline substances have been obtained which were found to be inhibitory to wheat and cowpeas, respectively, when these were grown in water solutions of these substances.^b These substances could not be obtained from these soils before the repeated growth of wheat and cowpeas.

The third proposition has to do with the constituents of plants that on the death and decay of vegetation become part of the soil, in contradistinction to those which may possibly be excreted during the growth of the plant. The number of such compounds which have been studied with respect to their toxicity to plants when compared with the number which may possibly find their way into the soil is very small, and no sweeping conclusions can be drawn as to what classes of compounds are and what are not inhibitory to plant growth. The work done, however,^c is sufficient to establish the fact that many such constituents are harmful. In view of the great number of such compounds, this work might be continued almost indefinitely, but since many of these plant constituents are undoubtedly soon changed into other compounds in the decay which dead vegetation undergoes in the soil, such continuation does not seem to be the best way in which to attain the end which is the object of fertility studies, viz, profitable rotation and rational fertilization. Once it has been established that some plant constituents are harmful, the attainment of this end seems to lie in the study of such bodies as are actually in the soil under infertile conditions and the way in which they arise in the soil.

To the three general statements made above there may be added, as a supplementary proposition, that the harmful compounds in soils

^a Bul. No. 40, Bureau of Soils, U. S. Dept. Agr.

^b Schreiner and Sullivan, Science, **27**, 329 (1908).

^c Bul. No. 47, Bureau of Soils, U. S. Dept. Agr.

which render them infertile or "exhausted" are organic compounds. There are, of course, soils which are infertile owing to the presence of excess of mineral salts, such as common salt, alkaline carbonates or sulphates, ferrous compounds, etc., but where such is the case the cause is generally easily discernible. With conditions such as these the present studies do not deal. By inhibitory or harmful soil compounds is here meant that residue of the complicated processes of plant life and plant decay which becomes an integral part of the soil; which causes the yield, when the same crop is grown continuously on some soils, to become less and less; which often renders the application of fertilizers necessary in the presence of a sufficiency of mineral plant food; which often compels the abandonment of the crop best suited to climate or market, or else forces a rotation with a less remunerative crop; which prevents the chemist diagnosing the cause of soil infertility by any of the numerous methods of analysis in use; which has rendered of no avail many elaborately planned plat experiments; and which is the cause of a natural succession of wild vegetation not explained by any change of soil or climate.

The evidence that the inhibitory bodies which cause infertility are organic compounds is both direct and indirect. As indirect evidence we have the absence of harmful inorganic material; the ready changes in many cases from harmful to nonharmful form by oxidation, whether by the roots of growing plants or by cultivation; the similar change effected by the addition of chemicals, both fertilizer salts and others; the change in injurious properties in many cases on heating, and in other cases the carrying over of the harmful properties on distillation, or removal by burning. Finally, the fact that the roots of growing plants possibly excrete harmful material which behaves as organic matter, and the fact that many organic plant constituents which find their way into the soil are inhibitory to plants, make the conclusion as certain as one based on indirect evidence can be. As final proof that there are in soils organic bodies harmful to plants, there is, as will be shown in the sequel, the isolation and identification of organic bodies from the soil which are inhibitory to plant growth, and which behave in solution as does the extract from an infertile soil.

The end sought in this study of soil fertility is a better understanding of the causes of infertility, and through this the removal, amelioration, or prevention of those bad soil conditions that may be the proximate causes of infertility, even when the real causes are the compounds formed under such conditions.

STATEMENT OF PROBLEM.

The extension of the investigation beyond the generalizations mentioned above involves, among others, three problems, each complex in itself. These are the identity and nature of the harmful substances in soils, their sources or the way in which they get into the soil, and finally the relation of harmful substances to each other, to nontoxic or even beneficial substances, and to added bodies such as fertilizers, both organic and inorganic.

These problems, especially the last stated, are very complex, and it would seem most improbable that the practice of agriculture could ever become scientific, except through the complete clearing up of the questions involved. Every effort should be made, then, to shed light on the identity, source, and general properties, including destruction or alteration, of inhibitory compounds. It is only by this that the practice of agriculture can be placed on the same plane with other great industries which have profited so much by scientific research in the past, a scientific development in which agriculture has not shared to its fullest extent.

With the first of the problems stated, the isolation, identification, and general properties of harmful soil compounds, the present paper is chiefly concerned.

The questions which naturally arise on a first approach to this problem are, first, what is the origin of the organic matter in general in the soil, what kind of substances furnish the material out of which the organic portion of soils is made, and what changes or transformation does it undergo in the soil? Second, are any of the organic compounds which first inquiry may have shown to become part of the soil, or to be formed in the soil, harmful to plants in the small amounts in which they would probably be present?

If in pursuit of this second inquiry it is found that some of the substances which get into the soil, or others which we must conclude are formed in the soil, are harmful, a further question arises. Have any of these substances been found in soils, or may there not be in the soil other substances which, because of insufficient data, have not been considered at all up to this point? The possibility of the presence in soils of compounds, of which neither the presence nor effect could be predicted, or even suspected, at once makes the inquiry the more general one, what do we know of the organic matter in the soil? The problem of the isolation, identification, and study of the general properties of organic soil compounds is, as has been stated, a complex one not only in itself but in its relations to other problems in soil fertility.

SOURCES OF THE ORGANIC MATTER OF SOILS.

An essential difference between soil and a mere mass of sand or disintegrated rock is that the former contains some organic matter. The first step in the transformation of bare rock into soil must be accomplished by the lowest forms of plant life, which obtain the materials necessary for their growth and reproduction from the air, rain water, and mineral matter dissolved from the rock. The death of these furnishes organic matter which can be utilized by higher forms, and thus the process goes on until there is an accumulation of organic matter sufficient, with the disintegrated rock material, to be called an arable soil and furnish a medium suitable for the growth of higher plants and agricultural crops. When the process of soil formation has reached this stage the number of micro-organisms, which at first were probably confined to a few species, is greatly increased, including bacteria and fungi of widely varying habits of growth and varied products resulting from this growth. There is, then, entering into the organic matter of a soil on which wild plants or crops are growing the remains of such parts of these plants as die and any products from the living plants. Still other compounds result from these through the growth of micro-organisms, by the oxidizing action of plant roots, by cultivation or lack of it, and by changes effected by fertilizers. Finally there are remains of micro-organisms themselves. It is probable that animal remains, such as insects and worms, should be accorded a more prominent place as a source of soil organic matter than is usually given them, for the amount of such material returned to the earth is very large. Earthworms are also an important factor, for they not only furnish animal remains, but much of the organic matter of plant origin is changed by passage through their digestive tract.

The final result of these complicated processes, it is hardly necessary to say, must be a mixture fully as complex as the processes through which it arises, and even when the original material has been similar in character the organic residue may be very different under varying conditions of temperature, moisture, aeration, cropping, and the character of micro-organisms present.

It is usual to consider the organic matter of both plants and animals as made up of protein, fats, and carbohydrates, but in addition to these elements there are, particularly in plants, a host of other compounds not included in these groups and which are a source of no small part of the soil organic matter. Among these are resins, hydrocarbons and derivatives, waxes, alkaloids, glucosides, tannins, phenols and their derivatives, acids, aldehydes, etc.

When the complex molecules of proteins, fats, and carbohydrates break down into simpler bodies they split along the same lines

of cleavage, depending on the structure of the original molecule, be it through the agency of micro-organisms as in decay, the agency of enzymes in digestion or agents such as acids or alkalies in the laboratory. These simpler bodies, which are termed primary cleavage or degradation products, are no doubt formed in the soil as elsewhere as the first steps of decay.

These primary cleavage products, however, particularly in the case of protein, are subject to still further decomposition through the same or other agencies. These secondary products are very numerous and of widely varying composition, structure, and, of course, chemical and physiological properties. To this is due the very different final products of decay or putrefaction under different conditions. To this variation in secondary decomposition products under different conditions of temperature, moisture, aeration, etc., the varying character of the organic matter in soils is probably due more than to differences in the plant or animal material which originally goes into the soil.

The number of such secondary products that may be formed is very large, and includes gaseous products which are the final stages of the decomposition of the organic matter into its elements, and practically all intermediate compounds between the original complex molecules and the final simple compounds. In other words, there is a continual "building down" process from the original complex molecule to simpler ones and these again into still simpler molecules, and so on down to the elemental stages of matter.

In addition to these various decomposition products in the soil, there are compounds which are built up by micro-organisms from the simpler degradation products. This reversal of the decomposition of the original organic matter into simpler compounds, by synthetic processes effected by micro-organisms, or possibly other agents, adds materially to the complexity of the problem.

Among the unclassified organic compounds spoken of above, such as resins, terpenes, tannins, etc., are some which are very resistant to decomposition by the agents active in the soil, and these may often exist in the soil in their original condition, at least for a time. On the other hand a great many break down into simpler bodies, as do the proteins and carbohydrates.

We have, then, as possible organic compounds in the soil, all plant constituents, compounds of animal origin, their degradation products, both primary and secondary, and also other products built up from these. Any attempt at complete enumeration of these would simply result in a long list of organic compounds with almost every class of such compounds known to science represented, and mention will be made here of but a few of the more prominent ones. Proteins, which are present in all parts

of living plants, are complex compounds the constitution of which has not yet been established. These in decay, as has already been stated, break down into simpler bodies, most of which have been thoroughly studied and their composition and constitution well established. Among these simpler bodies, which are nearly all nitrogenous, may be mentioned glycocoll, asparagine, alanine, leucine, phenylalanine, tyrosine, arginine, histidine, and lysine. Fatty acids may be present in plants either as free acids, in combination with glycerol as glycerides, or as part of the more complex molecule of lecithins. Stearic and oleic acids are present in some of these forms in nearly all plants, and there are in addition to these a large number of other fatty acids the distribution of which is in many cases limited to a few related species of plants. The composition and constitution of these is known in most cases, but with the exception of lecithins these fatty bodies are not so readily subject to breaking down in decay as are the proteins, and there is much less known of the products of such breaking down. The lecithins in breaking down give rise also to another class of bodies—choline, neurine, and betaine—all being well-known definite organic compounds of a basic nature.

The numerous substances formed during plant growth which are generally regarded as nonessential in the economy of the plant furnish a large amount of soil organic matter and include a large number of well-defined organic compounds. Among these are the alkaloids, terpenes, phenols, vegetable acids, and derivatives of these bodies. The composition and constitution of many alkaloids is well established; a large number being pyridine or quinoline derivatives, readily break down into simpler bodies like the pyridine or quinoline carboxylic acids, themselves well-defined compounds. The terpenes constitute another class of this kind, being widely distributed and including a large number of compounds, the composition and constitution of which is well known. Pyrocatechol, vanillin, cumarin, coniferin, and vegetable acids such as oxalic, citric, tartaric may be cited as examples of other well-known plant constituents.

It is evident from this brief survey of this phase of the problem that although the organic matter which goes into the soil is of an exceedingly varied and complex character, and that which forms in the soil may be even more so, there is considerable definite information available regarding the character of some of it. A great number of plant constituents, as well as the bodies to which they give rise on decay, are well-known definite chemical compounds. Some of these substances it requires no argument to prove get into the soil on the death of the plants containing them; others may be assumed to be formed in the soil. The question now arises, are any of these harmful to plants in the comparatively small quantity in which we might expect them to be present in soils?

EFFECT OF CERTAIN ORGANIC COMPOUNDS ON PLANTS.

There is in the literature considerable information regarding the injurious effects of various organic compounds on fungi, bacteria, algæ, and some of the higher plants, but for the most part these investigations have been purely physiological and have had no reference to, or direct bearing on, agricultural crops or soil studies. Either the compounds tested have no soil interest, the plants were lower forms, or the measure of inhibition or injury was based on some special function of the plant and not the general one of plant development.

It is not difficult to account for the dearth of data on the injury from organic soil compounds. The prominence given the mineral plant food theory of Liebig and the exclusion of almost every other consideration in dealing with the unproductiveness of soil brought about such a condition that from Liebig's time to the present the idea that organic compounds inhibitory to plant growth might be factors in the infertility or exhaustion of soil had practically no place in the philosophy of agricultural chemists.

Practically all that is known regarding the harmful effect of the organic compounds which get into the soil, or may arise in the soil, has been presented in another bulletin.^a The most important point—at least in this connection—established by the investigations there reported is that many of the possible organic soil compounds are harmful to plants, some in very small amounts.

From these investigations no general conclusions regarding the connection between effect on plants and chemical constitution were drawn, nor are the data sufficient to form the basis of any complete classification of such bodies. However, the establishing of a relation between effect and chemical constitution was not the object of these investigations, but rather to determine whether some plant constituents which get into the soil or compounds which may arise from them in the soil, are harmful to plants, in small amounts. This has been abundantly proven. It is also a matter of everyday observation that material containing many of the compounds found harmful is constantly being added to the soil. Many of the protein decomposition products, such as asparagine and leucine, not only are not harmful but appear to act as plant nutrients; others again, such as tyrosine, are injurious in quite small amounts. Choline was found to be harmful only in relatively high concentration, while the closely related neurine was injurious in concentrations very much less, and betaine, also closely related to both, was innocuous. The most markedly harmful bodies were found among those deposited within the plant but apparently not a

^a Bul. No. 47, Bureau of Soils, U. S. Dept. of Agr.

part of the life of the plant. Arbutin, vanillin, cumarin, heliotropine, esculin, terpenes, all of which are found to be very injurious, belong to this class.

Although there may be many plant constituents which are harmful to plants, and although it is evident that many such plant constituents must get into the soil on the death of vegetation, it would seem probable that some of them would be soon changed or destroyed by agents active in the soil so that they could not properly be considered part of the soil nor as factors in soil fertility. Others, however, might persist, since they are likewise strongly poisonous to lower forms of life and not readily oxidized for a longer or shorter period of time under certain soil conditions. This brings up another inquiry. Have any of these plant constituents or known derivatives, which are injurious and which are known to get into the soil, actually been separated from the soil? May there not be in the soil harmful organic compounds, the presence of which is not suspected or can not be predicted? These two questions involve a discussion of our knowledge of soil organic matter, first regarding the presence or absence of known compounds and second as to the possible presence of other compounds which could not have been suggested by the knowledge of the origin of soil organic matter.

PREVIOUS ATTEMPTS TO ISOLATE ORGANIC SOIL COMPOUNDS.

The first part of this inquiry can be very shortly disposed of. There is no record in the literature of any of these plant constituents that must get into the soil, or of any compounds that might be expected to be formed from them in the soil, ever having been isolated from any soil. This can not, however, be taken as evidence that these may not be present, for there is also no record that any individual members of this class have been especially or consistently sought for. There are, moreover, as will be shown in subsequent pages, such difficulties involved in this search that in many cases failure to find, by methods commonly used, would not be proof of their absence. The second phase of this question necessarily involves a somewhat full survey of the literature bearing on the subject and an examination of the claims to recognition of compounds described and named in the literature. This survey will serve as a guide in a systematic search for such soil compounds as are harmful to plants and, moreover, is essential to a thorough understanding of the complexity of the problem in hand and its relation to other problems, and furnishes a basis for attacking this complex problem with a reasonable hope of solving some of the intricate questions of soil fertility.

SEPARATION OF SO-CALLED HUMUS BODIES.

Probably the majority of agronomists, soil chemists, and students of soil fertility in general associate soil organic matter with the term "humus" and with humus only. With some there is the assumption that, when speaking of a soil, humus and organic matter are synonymous, and that any organic matter in the soil not humus is simply the undecomposed remains of plants and animals which have not yet reached the stage of decomposition where they can be properly considered a part of the soil. With others there is the assumption that, although there may be organic matter in the soil not humus, it is of such composition or form that it plays no part in the fertility of the soil, having a purely physical effect. Neither of these assumptions is warranted, as will be shown.

The term "humus," as originally used, had no especial agricultural or scientific significance, being simply another name for dark-colored vegetable mold and later applied to this mold material as a constituent more or less of all soils.

The term has now a more restricted meaning, especially as used by agricultural chemists, being applied more exclusively to the dark-colored organic matter extracted from soils by dilute solutions of sodium or ammonium hydrate and often called "humic acid." It is generally conceded, even by those prone to regard humic acid and allied acids as definite organic bodies, that the organic extract so obtained is a complex mixture and the method of determining humus in use to-day, which is essentially that of Grandeau,^a is merely an empirical method of determining a portion of the organic matter in the soil. Klaproth^b or Thomson^c applied the name "ulmin" to dark-colored amorphous bodies such as those obtained by Vauquelin^d from the bark of diseased elm trees. Sprengel,^e who obtained similar bodies from soils applied to these the name "humic acid." In 1838 Berzelius,^f evidently with the general meaning of the term "humus" in mind, used the term "humin" in describing certain dark-colored constituents of vegetable mold. Following this use of the term "humin," as applied to what was considered to be a definite organic body, a number of other workers took up the study of similar bodies and a number of other terms more or less related soon appeared. This group of workers included

^a *Traité d'analyses des matières agricoles*, p. 148, Paris, 1877. Bul. No. 107, Bureau of Chemistry, U. S. Dept. Agr., p. 19.

^b De Candolle, *Pflanzen-Physiologie*, I, 279 (1833).

^c *Jour. prakt. Chem.*, **21**, 325 (1840).

^d *Ann. Chim.*, **21**, 39 (1797).

^e *Bul. Soc. Chim.*, **10**, 173; De Candolle, *Pflanzen-Physiologie*, I, 280 (1833).

^f *Poggendorff's Ann.*, **44**, 375 (1838).

Braconnot,^a Mitscherlich,^b Boullay,^c Malaguti,^d and Mulder.^e The terms applied to humuslike bodies which have appeared more or less in the literature from that time to the present are associated for the most part with Mulder's name. Mulder had very definite ideas regarding the chemical individuality of these bodies. For instance, he says:^f

At present seven different organic substances are known to exist in the soil. They are crenic acid, apocrenic acid, geic acid, humic acid and humin, ulmic acid and ulmin.

These bodies he divided into two groups, one group consisting of crenic and apocrenic acids, the other group embracing all the others. As has been said, many of these terms, particularly humic acid, have come down through the literature of agricultural chemistry to the present and are now used with some writers at least with the same faith with which Mulder used them. According to Mulder these seven organic bodies found in soils were intimately related,^g and five at least were five distinct steps in the decay of organic matter in the soil. He regarded ulmic acid as the first step in this decay; this on further oxidation yielded humic acid and this in its turn, on still further oxidation, geic acid. Continued oxidation produced apocrenic and finally crenic acid. He ascribed to these the following formulas:^h Ulmic acid, $C_{40}H_{14}O_{13}$; humic acid, $C_{40}H_{12}O_{13}$; geic acid, $C_{40}H_{12}O_{14}$; apocrenic acid, $C_{48}H_{12}O_{24}$; and crenic acid, $C_{24}H_{12}O_{16}$.ⁱ

All this reads as though the matter were very simple, but the truth is that, although Mulder may have been in many ways in advance of his time, he was not able at a time when organic chemistry was in its infancy to clear up in so simple a manner one of the most complicated problems this branch of science has to deal with, viz, the composition of the organic matter in the soil.

Early investigators, including Mulder, soon found that sugar, starch, carbohydrates generally, and even albuminous bodies, when treated with strong acids or alkalis gave rise to dark-colored compounds having the same general appearance and properties as the humus bodies arising in the soil through decay. The same names were applied to these compounds and there was a general assump-

^a Ann. Chim. Phys., **12**, 191 (1819).

^b Lehrbuch, 3rd edit., I, p. 534.

^c Ann. Chim. Phys., **43**, 273 (1830).

^d Ann. Chim. Phys., **59**, 407 (1835).

^e Jour. prakt. Chem., **21**, 203, 321 (1840); **32**, 321 (1844).

^f The Chemistry of Vegetable and Animal Physiology, trans. by Fromberg, 1849, p. 146.

^g Loc. cit., p. 150.

^h Loc. cit., pp. 150 and 162.

ⁱ These formulas are on the old basis and to be modernized the hydrogen should be doubled.

tion that those in the soil and those produced in the laboratory were the same. As the number of investigators increased there soon arose disagreements regarding the composition of some of these, particularly humic acid, from several sources, it being found that the carbon content of these different preparations varied quite widely. Mulder attempted to reconcile such conflicting figures by showing that there were several kinds of humus and humic acids different in their character and fixed in their composition.^a

He held that the different humic acids might have a different composition due to the presence of one or more molecules of water so held that it could not be driven off at 140° C. For instance, he gives the following formulas:^b

Humic acid from sugar: $C_{40}H_{12}O_{12}$.

Humic acid from hard turf: $C_{40}H_{12}O_{12} + 3HO$.

Humic acid from an arable soil: $C_{40}H_{12}O_{12} + 4HO$.

One of the points of issue between these early investigators was whether humic acid and allied bodies contained nitrogen as a component. Mulder held that nitrogen was not a component of these bodies but was present as ammonia, that is, the acids were present in the soil as ammonium salts,^c and in this connection he says:

In a good arable soil—that is, one in which the organic constituents are as far as possible decomposed—none of these substances contain nitrogen as a constituent element; all their nitrogen exists in the state of ammonia.

Detmer came to the opposite conclusion^d on this point, claiming that the nitrogen in humic acid as obtained was present in organic combination. His conclusion was based on the fact that his humic acid, containing nitrogen, when treated with potassium hydrate gave off no ammonia, and only a small amount of nitrogen was given off in treatment with alkaline hypobromate. Later, however, Detmer stated that humic acid could be purified and obtained nitrogen free.^e

He gave for the acid the formula $C_{60}H_{54}O_{27}$ and also gave the following formulas for salts: $C_{60}H_{46}O_{27}Ca_3(NH_4)_2$, $C_{60}H_{46}O_{27}Fe_2(NH_4)_2$, $C_{60}H_{46}O_{27}Ag_8$.

From time to time there have been reported since the preliminary work outlined above, descriptions and formulas for humic acid and related bodies, the net result being about as many formulas as investigators, the only agreement being a general one of source, preparation, and somewhat vague properties.

^a Bul. Sc. Phy. et Nat. Neerl., 1840, pp. 1-102.

^b The Chemistry of Vegetable and Animal Physiology, p. 154. Formulas on the old basis of O=8.

^c Loc. cit., p. 146. Chemie der Ackerkrume, II, 256 (1862).

^d Landw. Vers.-Stat., 14, 248 (1871).

^e Jahresb. Fortsch. Chemie, 1873, p. 844.

Ulmic acid, according to Malaguti ^a had the formula $C_{12}H_{12}O_6$. Stein ^b ascribed to the humuslike bodies obtained by the action of hydrochloric acid on sugar the formula $C_{24}H_{18}O_9$. Lefort ^c obtained from rotten wood a humic acid to which he ascribed the formula $C_{24}H_{13}O_{17}$. Liebmann and Lettmayer ^d obtained what they claimed was the ammonium sodium potassium salt of humic acid. The humic acid prepared from this by precipitation contained carbon, 53.6 per cent, and hydrogen, 4.9 per cent. Thenard ^e prepared what he claimed was pure humic acid free from nitrogen and ash and gave the formula $C_{24}H_{10}O_{10}$. Mayer ^f obtained from Dopplerite a humic acid having the formula $C_{17}H_{18}O_8$. Demel ^g described the calcium salt of a humic acid having the formula $C_{24}H_{22}CaO_{12}$. Conrad and Guthzeit ^h obtained by action of acids on carbohydrates a humic acid containing 62.3 to 66.5 per cent carbon and 3.7 to 4.6 per cent hydrogen. Hoppe-Seyler ⁱ described humic acid obtained from brown coal which had the formula $C_{26}H_{22}O_{10}$; but as the barium salt showed the composition $BaC_{26}H_{22}O_{11}$ he considered the body obtained as the anhydride of an acid $C_{26}H_{24}O_{11}$. Berthelot and Andre ^j by the action of hydrochloric acid on sugar obtained a humic acid to which they gave the formula $C_{18}H_{16}O_7$. This, they claim, when heated to 110° C. gave an anhydride $C_{18}H_{14}O_6$. They further described the properties of this body ^k and stated that it was of the nature of a lactone.

As has already been stated, the object in reviewing the literature on this soil organic matter is for the purpose of arriving at some conclusion regarding the availability of such information as may be found there as a guide in a systematic search for such soil compounds as are harmful to plants. In other words, the chief concern at this stage of the present inquiry is the names and composition of humus bodies as definite organic compounds and their claim to recognition as such, rather than the broader question of the source and method of formation of such bodies and their relation to other bodies in the soil.

Leaving discussion of the results obtained by the workers quoted for subsequent pages, it is not out of place here to note that the most conspicuous feature of this work is the discordant results obtained for bodies bearing the same name and often from the same source.

During the period when the early work on humus bodies was being carried on, there was begun an investigation along another closely

^a Ann. Chim. Phys., **59**, 407 (1835).

^b Ann. Chem., **30**, 84 (1839).

^c Zeitsch. Chem., 1867, p. 669.

^d Ber. chem. Ges., **7**, 408 (1874).

^e Jahresb. Fortsch. Chem., 1876, p. 878.

^f Landw. Vers.-Stat., **29**, 313 (1883).

^g Monatsh. Chem., **3**, 763 (1883).

^h Ber. chem. Ges., **19**, 2850 (1886).

ⁱ Zeitsch. physiol. Chem., **13**, 109 (1889).

^j Ann. Chim. Phys., **25**, 364 (1892).

^k Compt. rend., **141**, 433 (1905).

related line, although at first sight this close relation might not be apparent. This was the study of the coloring matter in urine, either under normal or pathological conditions, or produced by chemical treatment.

The early work on this subject was, as might be expected, rather crude, and each investigator obtained different results and gave different and sometimes somewhat fanciful names to what they regarded as definite compounds. Udránszky in 1887 gathered together the loose ends left by these investigators and as a result of his own work stated that the darkening of urine on boiling with acids was due to a humuslike body formed from the reducing substances in the urine.^a Following this came the work of Hoppe-Seyler^b and Schmiedeberg^c along closely related lines and it soon came to be recognized by physiological chemists that plant or animal tissues or fluids, if they contain a carbohydrate group, either free or as part of a more complex molecule, will, if heated with strong acids, give rise to bodies of a humus nature. Schmiedeberg drew attention to the fact that the humus bodies so formed resemble in appearance, properties, and composition the bodies known as melanins and proposed for the artificially prepared bodies the name melanoidins.

The melanins are dark-colored amorphous pigments found in hair, skin, and the choroid coat of the eye and in pathological tissues such as sarcoma. They always contain carbon, oxygen, and nitrogen, and generally either iron or sulphur or both. They all agree in high carbon and low hydrogen content, but otherwise the composition varies according to the source. They do not give any of the reactions for proteins, nor so far as known any other special reactions. They are soluble in alkalis and are precipitated from such solutions by acids. In this, in general composition, color, and the absence of any distinctive reactions, the resemblance between the melanins and the humus bodies of the soil lies. The decomposition products of the melanins have been studied especially by Zdarek and Zeynek,^d Berdez, Nencki and Sieber,^e and Hoppe-Seyler,^f but it is thought by some that the derivatives found by these may have been impurities in the original material examined. In any case there is at present no knowledge of the constitution of these bodies, nor even is it known whether they are mixtures or a group of closely related bodies. Spiegler^g

^a *Zeitsch. physiol. Chem.*, **11**, 537 (1887); **12**, 33 (1888).

^b *Zeitsch. physiol. Chem.*, **13**, 66 (1889).

^c *Archiv. exper. Path. u. Pharm.*, **39**, 1 (1897).

^d *Zeitsch. physiol. Chem.*, **36**, 493 (1902).

^e *Archiv. exper. Path. u. Pharm.*, **20**, 346, 362 (1885); **24**, 17 (1888).

^f *Zeitsch. physiol. Chem.*, **15**, 179 (1891).

^g *Hofmeister's Beitr.*, **4**, 40 (1903).

has pointed out that the melanins may show quite different chemical reactions from the melanoidins.

The identity of melanins or natural pigments of animal origin with melanoidins or humus bodies is as yet undetermined, as might well be the case with what, so far as our present knowledge goes, may be mixtures of several amorphous bodies. The fact remains that these two groups of bodies are evidently closely related, and this convergence of two separate lines of investigation, one purely physiological and the other agricultural, till they meet in bodies closely related, if not identical, is of great interest.

Recent work on humus bodies is not very extensive, but what there is tends largely to discredit the claims of the bodies described by earlier workers to recognition as definite chemical compounds. For instance, Post^a and Muller^b point out that the humus bodies obtained from soils are often the chitin remains of insects, or the excrement of animals, and Korstyscheff^c that these humus bodies may often be remains of bacteria and molds. In 1904, Malkomesius and Albert^d published analyses of humic acids, so called, including the preparations of Merck and Krantz and a natural deposit known as Cassel brown. The figures obtained by them for hydrogen and carbon were approximately the same as those obtained by Detmer for humic acid from turf already quoted.^e Schermbeck,^f however, shows that the material analyzed and called humic acid by these authors was in large part a mixture of waxes and resins. As evidence of the variability of the so-called natural humus bodies the work of Cameron and Breazeale^g may be cited, showing that the carbon in humus obtained in the usual way from a number of typical American soils varied in carbon content from 33.3 per cent to 50 per cent.

Two papers have recently appeared presenting research work on humus bodies which are particularly valuable contributions to our knowledge of these bodies, in that the problem is attacked from new points of view, and by methods not heretofore utilized in the study of these bodies.

Suzuki^h reports results obtained by treating nitrogen-containing humic acid from different sources with boiling hydrochloric acid and then subjecting the solution obtained to esterification and fractional distillation according to the method of Fischer.ⁱ By this treatment

^a Landw. Jahrbücher, 1888, p. 405.

^b Studien über die natürlichen Humusformen, Berlin, 1887, p. 173.

^c Annales Agronomiques, **17**, 17 (1891).

^d Jour. prakt. Chem., **70**, 509 (1904).

^e Jahresb. über Fortsch. Chem., 1873, p. 844.

^f Journ. prakt. Chem., **75**, 517 (1907).

^g Jour. Am. Chem. Soc., **26**, 43 (1904).

^h Bul. Coll. Agr., Tokyo, **7**, 513 (1907).

ⁱ Zeitsch. physiol. Chem., **33**, 151, 412 (1901).

he separated and identified the esters of most of the monamino and diamino acids and other decomposition products obtained on boiling protein bodies with acids and in addition obtained a quantity of unidentified acids. The conclusion drawn from this work is that humic acid consists for the most part of an insoluble body of a protein nature and that amino bodies are not present as such. The latter part of this conclusion would seem not to be warranted by the work as stated. It is quite possible for some of the decomposition products which were obtained to have been present, though doubtless in smaller amounts, in the humic acid before treatment with boiling acids and to have escaped detection by the tests made. The work, however, does prove that the humic acid examined by him was either of protein nature, a mixture of protein decomposition products, or probably both, together with some as yet unknown bodies.

In a paper on the chemistry and physiological action of the humic acids ^a Robertson, Irvine, and Dobson report investigations into the chemical composition and constitution of humic acids. These authors obtained four preparations of humic acid—two natural, from peat by treatment with ammonium and potassium hydrate, respectively, and two artificial, by boiling sugar with hydrochloric acid and treating portions of the brown amorphous material so obtained with ammonia and potassium hydrate. They found some difference in composition of the natural preparations, that obtained through ammonia containing 54.24 per cent carbon, while that through potassium hydrate contained 56.67 per cent. The artificial preparations were almost identical in composition, except that the one obtained through ammonia contained 0.76 per cent nitrogen, while the other was nitrogen free. This nitrogen the authors speak of as combined, an assumption which, in view of the great absorptive power of humus bodies for ammonia, is not warranted in the absence of direct evidence of such combination. In these preparations the authors determined methoxyl, CH_3O , by the method of Zeisel^b and found such a wide difference between the natural and artificial preparations in this respect that the only conclusion justified is that there are important differences in constitution, despite the general agreement of properties and composition. In the humic acid from peat 1.71 to 2.47 per cent CH_3O was found, while in that from sugar 6.47 per cent was obtained.

Considerable space in this paper is devoted to a discussion of the empirical formula of humic acid, but the discussion is based on the assumption that the bodies obtained in the manner stated and called humic acid are definite organic compounds, an assumption which is, as will be shown, questionable.

^a Bio-Chem. Jour., 2, 458 (1907).

^b Monatsh. Chem., 6, 989 (1885).

DISCUSSION OF WORK ON HUMUS BODIES.

These results, obtained by investigations scattered over the last seventy years—results in many cases widely at variance with one another and viewed as a whole disconnected and fragmentary—are all that the student of the present day has presented to him in answer to the question, What do we know of humus organic matter in the soil? How much of this is true, how much erroneous, what shall be accepted and what rejected, are obviously questions that must be met at the outset. This has been met either consciously or unconsciously by all workers or writers in agriculture, in ways which may be summed up as follows:

A few agricultural writers accept some of the original names applied to humus bodies, notably humic acid, ulmic acid, and ulmin, and these names they use as though they were definite bodies of well-established composition and constitution. There is probably in the minds of these writers no definite idea of composition or constitution, or even any thought given to that side of the question, but the terms are used consistently and humates and ulmates are spoken of with the same certainty that one might speak of oxalates or citrates.

Other writers, the majority probably, if they have to speak of humus bodies as a class, state that they are probably complex mixtures of little-known bodies, but when they treat the broader subject of plant food and the function of humus in the soil they inconsistently speak of humates, ulmates, etc., with the same assurance as the first class.

A third class holds that the names applied by Mulder to humus bodies are names only; that the acid character of these so-called acids has never been demonstrated; and further, that there is no evidence that the bodies so designated are definite compounds, but rather in most cases that they are complex mixtures. The statement by Cameron and Bell^a puts the case concisely, "The existence itself of these acids has never been demonstrated * * * No satisfactory descriptions of the physical or chemical properties of these supposed acids, their salts, or characteristic derivatives, have been recorded," and this view can not be too strongly emphasized. Most physiological chemists, to whom the unknown constitution and uncertain composition of humus bodies or melanoidins is the most prominent characteristic, not to speak of the resemblance to melanins, also bodies of unknown constitution and variable composition, likewise concur in this view.

Very brief consideration of the status of organic chemistry at that early time and of the enormous advance made since then, an advance which has given nearly all knowledge included in that

^a Bul. No. 30, Bureau of Soils, U. S. Dept. Agr., p. 39.

branch of science at the present time, can not fail to convince one of the futility, not to say absurdity, of accepting unchallenged the findings of these early workers. Organic chemistry at that time was essentially elementary analysis—the determination of carbon, hydrogen, oxygen, and other elements—and observations on the ordinary physical properties of organic bodies. All other methods depended on at the present day for establishing the identity of organic compounds were unknown. The records of this early work give meager, if any, information regarding crystalline form of bodies themselves, or of their derivatives, and any mention of melting point or boiling point is lacking. All the present methods of addition, substitution, etc., by which organic compounds are arranged in classes according to structure were unknown or not recognized at their true value. Isomerism was not recognized and modern methods of molecular weight determination were not in use. It made no difference whether a body was amorphous or not, it was only necessary to have a mixture of bodies which remained somewhat constant through simple solution and precipitation, to find its percentage composition of carbon, hydrogen, and oxygen, record some of its common physical properties, and give it a name according to the fancy of the investigator, and it was established as a definite organic compound. If this body or mixture of bodies gave a precipitation with some mineral or alkaline earth, the precipitate was regarded as a salt and the organic mixture so precipitated was called an acid. Chlorophyll, protein, casein, gelatine—bodies regarding which our knowledge at the present day is very incomplete—were spoken of by Mulder and his contemporaries with the same certainty they assumed in regard to oxalic or citric acid, simply because they had all been subjected to elementary analysis and their common properties observed.

The striving of some of these early workers after simple relations between organic compounds based simply on elementary composition is one of the most prominent features of their work, and in this effort to classify compounds the statements they were led to make regarding bodies which we now know stand in no such relationship as they supposed is perhaps one of the best illustrations of the primitive nature of organic chemistry at that time, and a very convincing argument against accepting any of their work, unless verified in the light of our present knowledge. Speaking of vegetable acids, Mulder says:^a

Some acids exist in fruits, and it is worthy of remark that these bear all a simple relation to one another.

Citric acid, $C_4H_2O_4 \times 3 = C_{12}H_6O_{12}$.

Malic acid, $C_4H_2O_4 \times 2 = C_8H_4O_8$.

Tartaric acid, $C_4H_2O_5 \times 2 = C_8H_4O_{10}$.

Protartaric acid, $C_4H_2O_5$.

^a The Chemistry of Vegetable and Animal Physiology, trans. Fromberg, 1849, p. 805.

Again, in speaking of "gelatine sugar":^a

Sulphuric acid when acting on gelatine forms a kind of sugar containing nitrogen, represented by $C_8H_9N_2O_7$; if from this we subtract $C_6H_5O_5 = \frac{1}{2}$ equivalent of cane sugar, there remains $C_2H_4N_2O_2 = \text{urea}$,

a straining at a relationship between unrelated compounds which appears absurd to us now, but which is based on exactly the same kind of data and methods of reasoning as the relations stated to exist between ulmic, humic, and geic acids.

There is no reason to suppose that these early workers were not competent to perform elementary analyses, and the figures given for percentage composition may be received as accurate for the material analyzed, but there the accuracy stops. There is no reason for accepting the formulas given or for the assumption that the bodies examined are definite compounds. In fact, there is abundant evidence that such is not the case. There is, first, the amorphous, ill-defined nature of the bodies in question, not established as homogeneous by many modern methods available for such purpose; then the various and discordant figures for percentage composition obtained by different workers for what was supposed to be the same body; some evidence already quoted, obtained by workers of the present day, that these bodies really are mixtures; and, finally, very simple experimental evidence establishing this fact by which anyone can easily satisfy himself. If humus bodies be prepared from a soil in the usual way by extracting the soil with dilute alkali and precipitating these bodies from the solution so obtained by acid, there results, on thoroughly washing this precipitate, a body or mixture containing carbon, hydrogen, oxygen, nitrogen, and some ash ingredients. If this precipitate is again dissolved in alkali and precipitated again, there is obtained a mixture containing carbon, hydrogen, oxygen, and nitrogen in quite different proportions from the first precipitate. This can be repeated again and again, each time obtaining different results as to elementary composition. It is of interest to note in this connection that if this be repeated several times a point is reached where the humus precipitate becomes colloidal and passes through the filter. It is evident that by this procedure one could obtain as many humic acids as there were stages in the operation, although the composition varies less and less as one proceeds. In reality, the first precipitate is a mixture, and some of the bodies which seem to be held absorbed in this precipitate are removed by the subsequent operations and appear in the filtrates. This phenomenon is similar to that met with, for instance, in the analysis of the hydrochloric acid extract of a soil. In this case the bulky gelatinous precipitate of iron and alumina hydrates retain some of the lime, and this can not be removed by washing, but can be removed by dis-

^a Loc. cit., p. 233.

solving the precipitate in acid and reprecipitating. Again, if the first precipitate obtained as above, the so-called humic acid, be treated with solvents, such as alcohol or ether, it is easy to obtain therefrom bodies or mixtures of quite different composition and properties from the original mixture. Further, in the case of the so-called artificial humic acid, for instance, that produced on heating sugar with hydrochloric acid, the composition of the resulting dark-colored amorphous body varies quite widely as influenced by concentration of the acid or length of time of heating.

The only conclusion, then, that can be reached from this survey of the literature is that humic acid and allied bodies are mixtures of unknown compounds, and that if there is a single compound making up the major portion of the so-called humic acid, it is as yet of unknown composition, constitution, and properties, and that these bodies, whatever they may turn out to be, may be defined as those dark-colored amorphous compounds arising in soils through the decay of organic matter. In this group, then, would be included those soluble in alkalies, the so-called ulmic and humic acids, and also those insoluble in alkalies, known as ulmin and humin.

As doubtful as is the constitution or even composition of these supposed bodies, even more so is their action, harmful or otherwise, on plants, and no further discussion is called for by the purpose and scope of the present bulletin.

SEPARATION OF NONHUMUS BODIES.

The knowledge to be gleaned from a survey of the literature, of compounds other than these dark-colored mixtures discussed above is somewhat more definite in character. Crenic and apocrenic acid are the only nonhumus bodies recognized by Mulder and others of his day. These acids were first described by Berzelius^a and further described by Mulder. The status of these bodies is exactly that of humic acid and other soil bodies described by Mulder, and all that has been said regarding the one class may be applied to the other. There is no evidence that any such definite organic compound of the composition and properties described exist in soils.

The possibility of there being other organic nonhumus bodies than these was early recognized, and, as has already been noted, played a part in the early discussion whether humic acid contained nitrogen as a component. It was not, however, until comparatively recent times that anything definite appeared along this line, and all the information so far obtained and suggested centers around the organic nitrogenous compounds. Among workers who contributed to the literature on the question of the character of the nitrogen in humus bodies may be mentioned Loges,^b who found that a hydrochloric

^a Pogg. Ann., 29, 1 (1833).

^b Landw. Vers.-Stat., 32, 201 (1886).

acid extract of a soil contained a nitrogen-holding humus body which yielded a precipitate with phosphotungstic acid, and Eggertz,^a who found from analyses of thirteen samples of humus that the nitrogen content varied from 2.59 per cent to 6.43 per cent, and that it was present in organic combination, not as ammonia. This conclusion he based on the fact that the nitrogen could not be removed by repeated solution in alkali and precipitation with acids. The probability of the presence in soils of organic nitrogenous compounds not in combination with the humus apparently received very little attention until quite recently.

Baumann^b in 1887 suggested the presence of amino compounds in soils, basing the suggestion on the fact that on boiling soils with hydrochloric acid, ammonium compounds not originally present are formed. Berthelot and Andre^c concluded from their investigations that the nitrogenous compounds in the soil were chiefly insoluble amids which are easily split into ammonia and soluble amids. Warrington^d demonstrated the presence of small quantities of soluble amids in soils, and Sestini^e showed that at least a portion of the soil nitrogen was present as amino acids. Dojarinko^f obtained from Russian black soils large quantities of amino nitrogen. In 1905 Shorey^g published results obtained by the application of the methods of Housmann,^h as modified by Osborne and Harris,ⁱ to soil. The object of this method is a classification of the decomposition products obtained on boiling protein with strong acids and the results obtained indicate the presence of some of these decomposition products or compounds which give rise to them on boiling with acid. None of these investigations deal with the isolation or identification of any definite amino compound, but are merely attempts to classify a portion of the organic matter, and serve as an indication of what might be found in the soil, and neither the results obtained nor the methods used need be commented on here.

Considering now nitrogenous compounds not of an amino nature, Berthelot and Andre^j obtained results on the distillation of soils which suggested to them the presence of a pyridine compound.

^a Meddelanden fran Konigl. Landbruks-Akademiens Experimentalfält. Stockholm, 1888, pp. 1-66; Centralbl. f. Agr. Chem., 1889, p. 75.

^b Landw. Vers.-Stat., **33**, 247 (1887).

^c Compt. rend., **103**, 1101 (1886).

^d Chem. News, **55**, 27 (1887).

^e Landw. Vers.-Stat., **51**, 153 (1899).

^f Landw. Vers.-Stat., **56**, 311 (1902).

^g Agr. Invest. Hawaii in 1905, Report of Chemist, p. 34.

^h Zeitsch. physiol. Chem., **27**, 95 (1899).

ⁱ Jour. Am. Chem. Soc., **22**, 323 (1900).

^j Compt. rend., **112**, 598 (1891).

Recently Shorey^a has isolated from an Hawaiian soil a pyridine compound, picoline carboxylic acid, which appears to be the first isolation and identification of a definite crystalline organic compound from a soil.

Of the isolation of nonhumus compounds not containing nitrogen there is no mention in the literature except statements that humic acid, as originally obtained, often contains waxes and resins, and that compounds like acetic acid, formic acid, alcohol, etc., have been detected.

Summing up this survey of the literature, it might be stated that the most conspicuous feature is lack of specific knowledge concerning the organic matter of the soil. The humus bodies are a group of compounds of unknown composition and constitution, some of which seem to resemble the natural animal pigments known as melanins, themselves bodies of indefinite composition and unknown constitution, apparently of a protein nature. There is some evidence that amino compounds are present in certain soils, but beyond this there is no knowledge what members of this large group of nitrogenous bodies may be present. The presence of waxes, resins, and other difficultly decomposable plant residues is indicated, and finally there is the isolation of a single nitrogenous compound picoline carboxylic acid, which doubtless constitutes but a very small portion of the organic matter.

It may not be out of place to remark that this ignorance concerns an important soil ingredient, the presence of which makes soil out of what would otherwise be rock powder or sand. As has been stated in a previous bulletin,^b the amount of organic matter in ordinary soils is appreciably large, for as the result of analyses of several thousand samples of soil from all parts of the United States, Cameron^c has shown that the average organic content of soils is 2.06 per cent and of subsoils 0.83 per cent. If the soil be taken as 8 inches deep, there would be an average of 28 tons of organic matter in the soil per acre and 50 tons in the soil and subsoil to the depth of 2 feet.

There are, then, in most soils tons of organic matter, matter which is constantly being changed through the activity of micro- and macro-organisms, through cultivation and fertilization; matter which includes bodies harmful to vegetation, other bodies probably beneficial, and still other bodies wholly inert, but matter about which there is very little chemical knowledge.

^a Report Hawaii Agr. Expt. Sta., 1906, p. 37.

^b Bul. No. 47, Bureau of Soils, U. S. Dept. Agr., p. 10.

^c Jour. Am. Chem. Soc., **27**, 256 (1905).

EXPERIMENTAL METHODS.

The problem which the student of soil fertility has to face may now be stated tentatively as follows: Many soils are infertile because of the presence in them of organic matter inhibitory to crops. Of this organic matter, which is presumably relatively small in amount, nothing definite is known chemically. Not only this, but on a survey of the investigations into the nature of soil organic matter which have been carried on in the last seventy years it is found that there is nearly a total absence of the proof of the presence of any definite organic compound in the soil, either harmful or otherwise. How can some knowledge be obtained of the chemical nature and properties of these bodies, that there may be intelligent treatment of soils containing them, treatment that will bring about certain destruction or change of the harmful bodies, or prevent their formation, instead of the rule-of-thumb methods now in use. Obviously one way to attain this knowledge is to isolate these bodies from soils and study their properties.

To anyone who realizes the paucity of knowledge of soil organic matter, which has been emphasized in previous pages, it will be apparent that the isolation of any definite organic compounds from the soil is not an easy task. When the task is narrowed down to the isolation of injurious compounds, the difficulties are greatly increased. Before outlining the methods by which this problem has been approached, some of these difficulties should be considered, for the matter resolves itself into a choice of methods to surmount these difficulties.

It is evident, in the first place, that for any organic soil compound to be harmful to plants it must be present in the soil solution; that is, it must be at least slightly soluble. Investigations have shown that many organic compounds stated in the literature to be insoluble are soluble to the extent of a few parts per million at least, and sufficient in many cases to have a harmful effect on plants grown in the solution. The water extract of an infertile soil usually contains but a very small quantity of organic matter, and it is a reasonable presumption that the injurious organic compound is only a part of the total organic matter present. Such being the case, it is evident that the isolation of any organic compound from a water extract of a soil in sufficient amount to establish its identity and study its properties is in most cases a problem that does not come within the limitations of laboratory work. In other words, the amount of soil or soil extract necessary to handle would be too great for ordinary laboratory appliances and methods. This difficulty, due to the small quantity of organic material which must be present in the soil solution, is much increased by the fact that the inhibiting substances are un-

known compounds with unknown chemical and physical properties. In many respects the isolation of an injurious substance from an infertile soil and the detection in animal organs of a poison of which the identity, properties, characteristic reactions and symptoms produced are all unknown, are analogous.

With the water extract of a soil practically unavailable as a source of the unknown compounds under the present limitations of laboratory work, and with the lack of methods applicable to such research, the only course open is to make use of all other methods which are known or can be devised for the solution of the soil organic matter, and to attempt to isolate from such solution definite compounds, taking advantage of proven methods, or devising new ones.

Before stating the methods which have been used with different soils in the attempt to isolate from them organic bodies, and the results obtained thereby, there are a few facts regarding the soil organic matter which are true more or less for any soil and which should be kept in mind throughout the discussion of the subject. The organic matter in soils is generally but little soluble at ordinary temperatures in neutral solvents, such as alcohol, ether, or chloroform. Dilute acids at ordinary temperatures have also little solvent action on soil organic matter, and if heated there is usually a change effected in the constitution of some of the organic bodies. Dilute alkalies at ordinary temperatures dissolve relatively large quantities of soil organic matter; in fact, in the case of some soils, nearly the whole of the organic matter can be so dissolved by repeated treatments. This treatment of soils with dilute alkalies, although it may in some cases effect a change in the constitution of some of the organic compounds, especially those nitrogenous, seems at present the only method of effecting the solution of a large part of the organic matter with the minimum chance of changing the character of the compounds sought, and for this reason was the first method used in the attempt to isolate harmful organic bodies from the soil.

SEPARATION OF PICOLINE CARBOXYLIC ACID.

The soil first treated in this way was from Takoma Park, Md., and is fully described in a former bulletin.^a This soil, which is characterized physically by large percentages of clay, silt, and coarse sand, and very little of the intermediate material, is quite light in color and contains, contrary to what might be expected from its appearance, 3 per cent organic matter. It contains 0.1 per cent nitrogen, and analyses of both water and acid solutions indicate the presence of sufficient mineral plant food for ordinary crops. It is, however, an exceedingly

^aBul. No. 28, Bureau of Soils, U. S. Dept. Agr.

infertile soil and does not respond readily to treatment with either stable manure or commercial fertilizers. A bacteriological examination of a sample of this soil by Mr. Karl F. Kellerman, of the Bureau of Plant Industry, showed this to be rather low in bacterial content though active in denitrification.

The water extract of this soil is a poor medium for the growth of wheat seedlings, but is much improved by treatment with carbon black, by dilution, and by the addition of pyrogallol or calcium carbonate. In other words, field observations of this soil indicate that something other than lack of plant food is the cause of infertility, and laboratory observations confirm this and in addition indicate that this infertility is due to the presence of some harmful substance.

When the soil is treated with a 2 per cent solution of sodium hydrate there results a dark-colored solution, the so-called humus extract. If this solution after separation from the soil residue be treated with an excess of acid a flocculent dark-colored precipitate is formed, the so-called humic acid. The filtrate from this is dark colored but much lighter than the original extract and contains a portion of the organic matter. On bringing this extract to neutrality by the addition of sodium hydrate there is formed a further precipitate containing both organic and mineral matter. The neutral filtrate from this is still colored dark yellow and contains a considerable portion of the organic matter originally extracted from the soil by sodium hydrate. By dissolving both of the above precipitates again in sodium hydrate and repeating the operations of acidifying, filtering, neutralizing, and again filtering, there can be obtained a still further quantity of organic matter in neutral solution, which can be mixed with the first portion.

This process results in a neutral solution containing some of the organic matter and a comparatively large amount of a sodium salt resulting from the neutralization of the sodium hydrate used. This neutral solution of soil organic matter gives precipitates upon the addition of a number of mineral salts such as basic lead acetate, cupric acetate, mercuric nitrate, and silver nitrate, precipitates which contain organic matter and are usually nitrogenous.

In this method of obtaining a neutral solution of soil organic matter, the amount so obtained in solution seems to be approximately the same, whether hydrochloric, sulphuric, or nitric acid be used for neutralizing; acetic acid, however, usually gives a smaller amount in solution than the mineral acids. The final treatment to which the solution is to be subjected must decide what acid is used throughout, e. g., hydrochloric acid would not be used if there were any intention of using silver nitrate as a precipitant, nor sulphuric acid if lead subacetate was to be used.

METHOD OF ISOLATION.

The method finally adopted for this particular soil was to treat it for twenty-four hours with frequent shaking with 2 per cent solution of sodium hydrate free from chlorides, allowed to stand until the soil residue settled and the dark-colored solution siphoned off. To this was added a slight excess of nitric acid free from the lower oxides of nitrogen and the flocculent humus precipitate filtered off. The acid filtrate was then exactly neutralized by the addition of sodium hydrate and the precipitate so formed filtered off. To the resulting neutral solution was added a solution of silver nitrate and the precipitate formed, which was slight in amount, was allowed to settle, the clear liquor decanted, and the precipitate collected on a filter and washed with a small quantity of water. The precipitate while still moist was suspended in water and treated with hydrogen sulphide, the silver sulphide filtered off, and the filtrate evaporated to small volume on a water bath and allowed to cool slowly. There was thus obtained a more or less colored residue in which crystals formed. The crystals were separated from the mother liquor, washed with a small quantity of cold water, dissolved in hot water, and precipitated again with silver nitrate. Filtration, treatment with hydrogen sulphide, etc., being now repeated, the crystals were usually obtained fairly free from color and could be further purified by recrystallization from water.

PROPERTIES OF THE ISOLATED COMPOUND.

The crystals obtained are little soluble in cold water, easily in hot, little soluble in alcohol, and almost insoluble in ether. From water the compound separates in oblique prismatic crystals with water of crystallization which they lose at 100° C. In Plate I, figure 1, is shown a photomicrograph of these crystals obtained by allowing a rather dilute solution in hot water to cool slowly, and figure 2 shows the crystals obtained by cooling a rather concentrated solution in water. The latter crystals are usually very thin plates, superimposed, forming large scale-like masses and giving very brilliant color effects with polarized light. The substance sublimes unchanged on heating in an open dish and does not melt in a capillary tube at 300° C. A water solution is faintly acid to litmus, and when neutralized does not give a precipitate with either barium chloride, calcium chloride, cadmium sulphate, or lead acetate. Cupric acetate gives a bluish crystalline precipitate, insoluble in cold water. Silver nitrate gives a flocculent amorphous precipitate.

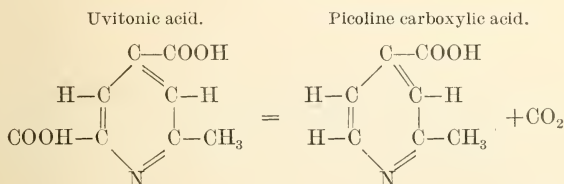
IDENTIFICATION AS PICOLINE CARBOXYLIC ACID.

The crystals have, therefore, the appearance and properties of picoline carboxylic acid, which, as stated, had been isolated by Shorey from a Hawaiian soil by the same method. The compound from Hawaiian soil was further identified as picoline carboxylic acid by its corresponding water of crystallization, its nitrogen content, the chlorine content of the hydrochloride, and its conversion into lutidinic acid on oxidation. The last-named acid is characterized by crystallizing in leaflets little soluble in cold water and melting at 239° C., and giving a yellowish or orange color with ferrous sulphate.

The preparation obtained from the Takoma soil having the properties stated above was compared with that obtained from Hawaiian soil, and also with picoline carboxylic acid prepared artificially in the laboratory, and found to correspond throughout.

PICOLINE CARBOXYLIC ACID AND RELATED COMPOUNDS.

Picoline carboxylic acid, which has heretofore been known as a laboratory product only, has been described by Böttinger^a and can be prepared by heating uvitonic acid to 274° C. Uvitonic acid, which is methyl pyridine carboxylic acid, breaks up at this temperature into carbon dioxide and picoline carboxylic acid, as shown below:



Uvitonic acid can be easily prepared in the laboratory by treating pyruvic acid with alcoholic ammonia, separating the precipitate formed, washing it with ether, dissolving it in water, and precipitating the free acid by the addition of sulphuric acid, as described by Böttinger.^b

Leaving the toxicity of picoline carboxylic acid for discussion in subsequent pages in connection with other substances found in soils, it may be said here that it was found to be not very harmful to wheat seedlings, while the nearly related uvitonic acid was quite so. While this slight harmful effect and the apparently very small amount of this body in the Takoma soil make it improbable that it is responsible for more than a small part of the infertility of that soil, its close connection with the uvitonic acid and other closely related acids,

^a Ber. chem. Ges., **14**, 67 (1881); **17**, 92 (1884).

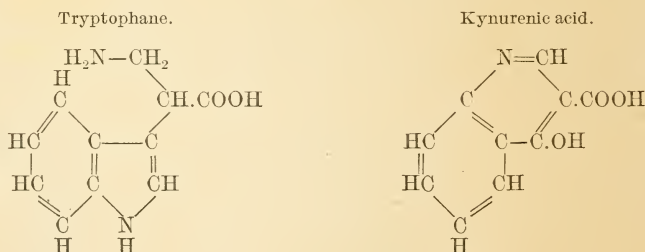
^b Ann. Chem., **188**, 330 (1877); **208**, 138 (1881); Ber. chem. Ges., **13**, 2032 (1880).

such as pyridine tricarboxylic acid,^a known to be antiseptic, justifies the suspicion that there may be present some other pyridine compound either a source of or a product of the one actually found and one which might be the active cause of infertility.

POSSIBLE ORIGIN OF PICOLINE CARBOXYLIC ACID.

Regarding the origin of the picoline carboxylic acid in the soil, very little can be said at the present time, yet it may not be out of place to call attention to a few facts in connection therewith. In the laboratory picoline carboxylic acid can be formed from pyruvic acid and ammonia through the intermediary compound, uvitonic acid, by splitting off carbon dioxide. Pyruvic acid has been shown by Mörner^b to be a constant secondary decomposition product of protein, and as such might be formed in the soil; in fact, there are some indications of its presence along with picoline carboxylic acid in the soil under investigation. Should this be the case, then it is not impossible that the remaining steps in the formation of picoline carboxylic acid should take place in the soil, although no reaction whatever for the uvitonic acid could be obtained. The most that can be said, therefore, is that the picoline carboxylic acid may possibly have arisen as the result of the splitting and decomposition of protein in the soil.

A further point of interest in regard to the formation of the pyridine nucleus in the soil through the decomposition of protein is shown by the formation of pyridine on reduction of the melanoidins or humin substances, resulting from the dissociation of protein, as demonstrated by Samuely.^c Hopkins and Cole^d have further shown that tryptophane, one of the dissociation products of protein, is readily changed to dark-colored humin substances on boiling with acids, or even with water, and Ellinger^e has suggested for tryptophane the structural formula:



which, as is readily seen, contains the possibility of the pyridine closure, as illustrated by its transformation into kynurenic acid. All

^a Rademaker, Med. Herald, **19**, 107 (1887).

^b Zeitsch. physiol. Chem., **42**, 121 (1904).

^c Hofmeister's Beitr., **2**, 355 (1902).

^d Jour. Physiol., **27**, 418 (1901); **29**, 451 (1903).

^e Zeitsch. physiol. Chem., **43**, 325 (1904).

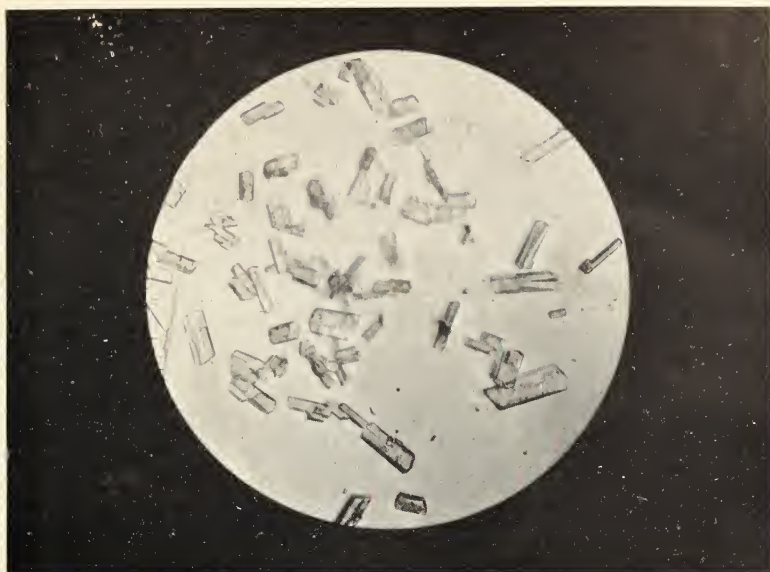


FIG. 1.—CRYSTALS OF PICOLINE CARBOXYLIC ACID OBTAINED FROM SOILS, FORMED FROM A DILUTE AQUEOUS SOLUTION.

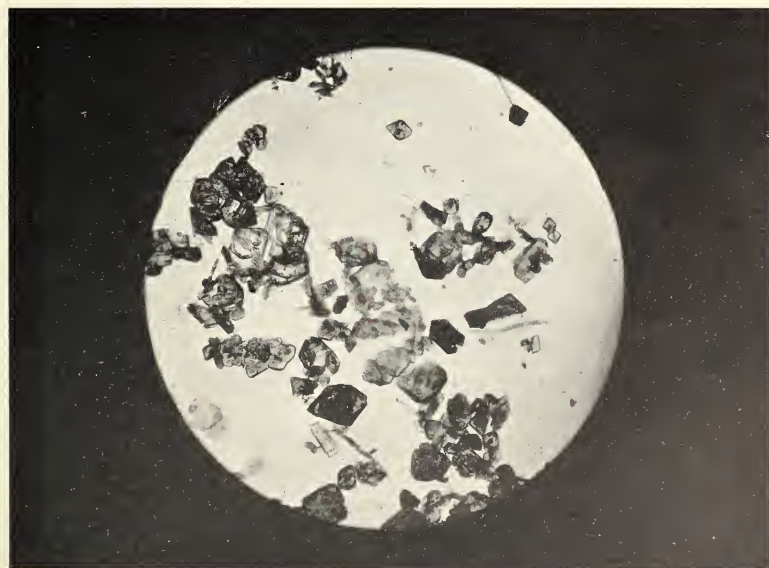


FIG. 2.—CRYSTALS OF PICOLINE CARBOXYLIC ACID OBTAINED FROM SOILS, FORMED FROM A CONCENTRATED AQUEOUS SOLUTION.



FIG. 1.—CRYSTALS OF DIHYDROXYSTEARIC ACID OBTAINED FROM SOILS.



FIG. 2.—CONCRETIONS OF THE BARIUM SALT OF DIHYDROXYSTEARIC ACID.

these facts indicate strongly that the origin of the pyridine ring in the soil, whether it be as picoline carboxylic acid or other pyridine derivative, lies most probably in the decomposition of protein bodies.

THE PRESENCE OF OTHER COMPOUNDS INDICATED.

There has been gathered some evidence of the presence of pyruvic acid in the Takoma soil, but this evidence is as yet indicative only. This evidence, stated briefly, is as follows: When the neutral soil extract obtained as outlined above is made alkaline with sodium hydrate and then a solution of iodine in potassium iodide added there is formed in a short time at room temperature a precipitate of iodoform. The same precipitate can be obtained by treating the original sodium hydrate extract of the soil with iodine reagent. The iodoform so obtained was identified, after recrystallizing, by its melting point and by its characteristic crystalline form as well as its odor. Pyruvic acid forms iodoform when treated in this way, but this is true of other acids, for instance, lævulinic. Again, when the neutral soil extract is made acid and treated with a solution of phenylhydrazine hydrochlorate there is produced in a short time a reddish-colored micro-crystalline precipitate which soon tends to become resinous. It was not found possible by any means so far tried to purify this precipitate so as to obtain any characteristic crystalline form or constant melting point. The phenylhydrazone of pyruvic acid crystallizes in needles and melts at 178° to 183° C. It is evident that if pyruvic acid is present there is also present another compound giving a precipitate with phenylhydrazine. Bülow^a has described a reaction of the phenylhydrazones which he states is characteristic. When the phenylhydrazone is treated with concentrated sulphuric acid and dichromate of potassium there is produced a purplish color. This color is produced by the phenylhydrazone precipitate from the soil extract and by the pure phenylhydrazone of pyruvic acid.

Simon^b has described a color reaction of pyruvic acid which he states is characteristic. This depends on the blue color given by sodium nitroprusside and ammonia when added to dilute solution of pyruvic acid. When the neutral soil extract is treated with these reagents there is produced a very slight greenish color, which, since the soil extract is orange colored, might be expected in the presence of small amounts of pyruvic acid. The delicacy of the reaction was tested by adding known quantities of pyruvic acid to the neutral soil extract and adding the reagents, and it was found that the reaction was not at all delicate in the presence of the other material present

^a Ann. Chem., **236**, 195 (1886).

^b Compt. rend., **125**, 534 (1897).

in the soil extract, as much as 500 parts per million being required to give the characteristic blue color, lower concentrations producing greenish shades. These reactions indicate at least the probable presence of pyruvic acid as well as other bodies not yet isolated.

Pyruvic acid was found to be quite injurious to wheat seedlings, but was not so when neutralized with sodium hydrate, and if present in the soil its activity as a factor in infertility would no doubt depend on the form in which it might be present. Apart from this the possible presence of this acid in the soil is of interest, for it may be that through the agency of ammonia in the soil the change from the open chain nonnitrogenous pyruvic acid to the ring form nitrogenous uvitonic acid could be effected, as is done in the laboratory with alcoholic ammonia. Uvitonic acid has been sought for in the various soil extracts, but so far with no indication that it is present.

When picoline carboxylic acid is prepared from the neutral soil extract by the method stated above there is separated from the crystals a dark-colored mother liquor containing nitrogen. When this is evaporated nearly to dryness there usually separates a further small quantity of picoline carboxylic acid, but no other crystalline body has been obtained or observed. The mother liquor at this stage, however, is thick and viscous and of such a nature that crystallization of any body present would be difficult. When the mother liquor is evaporated completely to dryness and then taken up again with water there is found a quantity of amorphous colored matter which does not redissolve.

When the dry residue obtained by evaporating the mother liquor to dryness is heated with caustic lime there is given off an odor of crude pyridine, indicating a still further quantity of picoline carboxylic acid or of some other pyridine compound. When the mother liquor from which picoline carboxylic acid has been separated is made alkaline with sodium carbonate and treated with a solution of diazo sulphanilic acid, a dark-red color is slowly formed. The color produced by this reagent with certain organic compounds was first noticed by Ehrlich^a and further described by Pauly,^b who found that it could be used for the detection of histidine in the absence of tyrosine. Histidine gives with this reagent a dark-red color which remains red on dilution, and no other protein decomposition product except tyrosine gives this color, all others giving yellow colors which disappear on dilution. According to Pauly the distinctive color given by histidine and diazo sulphanilic acid is due to the imidoazol group, histidine being α -amino β -imidoazol propionic acid. The color obtained by treating this soil material with diazo sulphanilic acid does not appear to be that obtained with histidine, being slower in appear-

^a Zeitsch. klinische Med., **5**, 285 (1882).

^b Zeitsch. physiol. Chem., **42**, 508 (1904); **44**, 159 (1905).

ing and soon losing the red tint on dilution. The same color can be obtained in the distillate on distilling the original soil with steam, and is much more pronounced if the soil be made slightly alkaline before distillation. Other organic compounds than those mentioned in the literature were found to give this color, for instance, arbutin^a and piperidine, so that the most that can be said of this reaction is that it indicates the presence of some body not yet isolated or identified.

SEPARATION OF DIHYDROXYSTEARIC ACID.

The comparatively slight harmful effect of picoline carboxylic acid, the only compound up to this point isolated from the Takoma soil, coupled with the apparently very small quantity present in this soil, led to the conclusion that there must be some substances of much more injurious character than this present—substances perhaps not in any way connected with the one obtained or others indicated. With the object of, if possible, obtaining such other bodies from this soil a number of other methods were tried.

METHOD OF ISOLATION.

One which finally gave definite results was as follows: The soil was treated with 2 per cent sodium hydrate solution as in the method first used. This alkaline extract was made acid with a slight excess of acetic acid and filtered from the humus precipitate. There was thus obtained a dark-colored extract containing organic matter, sodium acetate, acetic acid, and some mineral matter from the soil. This acid extract was shaken out with ether and the ether allowed to evaporate on the surface of a small quantity of water in a warm place. The ether on evaporating left on the surface of the water some colored resinous material, together with nodules or concretions of a crystalline body. On heating the water to boiling these crystals went into solution, while the resinous matter remained undissolved, and on pouring the solution into a filter previously wet with hot water the resinous material remained in the filter. On cooling the solution so obtained, crystals separated, which generally appeared as very small plates or leaflets arranged in radiating clusters. These if still colored could be purified by solution in hot water and filtering as before. This body, which is white when pure, melts at 98° to 99° C., is little soluble in cold water, somewhat readily in hot, and quite soluble in alcohol or ether. A water solution is acid to litmus and decomposes barium or calcium carbonate, forming the corresponding salts, which are difficultly soluble in cold water. This body was found to be quite harmful to wheat seedlings.

^a Bul. No. 47, Bureau of Soils, U. S. Dept. Agr.

ISOLATION FROM SEVERAL SOILS.

With the object of extending the research to other soils, six more or less infertile soils, selected from near Arlington, Va., Indianapolis, Ind., Center Hall, Pa., State College, Pa., Pulaski, Tenn., as well as that from Takoma, Md., were treated as follows: Eight hundred grams of each were treated with 2,000 c. c. of a 2 per cent sodium hydrate solution for twenty-four hours, with frequent shaking; after standing, 1,000 c. c. of the supernatant clear extract was treated with a slight excess of acetic acid and filtered. The filtrate was shaken out twice with ether and the ether allowed to evaporate on the surface of a small quantity of water, the water boiled and filtered, and the filtrate cooled. The character of any crystals which formed and the approximate amount was observed. From four of the soils examined there was obtained crystalline material having the same general appearance and properties as that obtained by this method from the Takoma soil as described above. The amount of this material obtained from 1,000 c. c. of the original extract was then dissolved in water and made up to 1,000 c. c. with distilled water and used as a culture medium for wheat seedlings. Dilutions of this stronger solution with one and with nine parts of pure distilled water were also used. The results obtained were in direct relation to the amount of crystalline material, the solution containing the greatest portion being the most harmful. The results obtained with one of the soils, that from Tennessee, are shown in Plate III, figure 2.

The soil from which, by this treatment, the most material was obtained and which furnished the most injurious medium was from Tennessee, a soil which had been in cultivation fifteen years, for the most part in cotton. This soil is classified as Clarksville silt loam, and although light colored, contains 3.26 per cent organic matter and 0.16 per cent nitrogen. The surface soil (which was collected) is a gray silt loam 6 inches deep. The subsoil to a depth of 3 feet is yellow silt loam, having a close, compact structure. The field is in a large area of this type, the Clarksville silt loam, occupying the broad, nearly level top of a ridge. There were a few apple trees in the field, but the sample was taken at some distance from any of them. The yields have been light. On an area about 50 feet square, from which the sample was taken, the plants were only about one-half to one-fourth as large as in other parts of the field, and matured very few bolls. Where vacancies occurred in the cotton rows, corn had been planted. The same difference existed in the corn as in the cotton. Near where the sample was taken the stalks were small, with a yellow color, and had failed to form good ears. In other parts of the field the corn had a good color and average-sized ears had been

produced. The east half of the field contains 20 apple trees, set five years ago. One tree stands about 30 feet from where the sample was taken and is smaller than the trees farther away, thus agreeing with the growth of the cotton and the corn in different parts of the field.

A water extract of this soil was a poor medium for the growth of wheat seedlings and was improved by treatment with carbon black.

Having, then, a soil unproductive in the field and in the laboratory, and yielding by simple treatment a crystalline body injurious to plant growth, the foundation was laid for the isolation of a harmful soil compound in sufficient quantity to determine its identity and study its properties. Large quantities of this soil were treated by the method already mentioned, extraction with 2 per cent sodium hydrate solution, acidifying with acetic acid, filtering from the humus precipitate, shaking out the acid filtrate with ether, and crystallizing the water-soluble portion of the ether extract from water. By this treatment, extracting each portion of the soil twice, the body described was obtained in a fairly pure condition in amounts of 0.05 gram per kilo of soil, which is 50 parts per million, or 200 pounds per acre to the depth of 1 foot. The quantity actually present in the soil must of course be much larger, for when all is said, the methods of extraction are very crude and far from quantitative, and the amount obtained in crystalline form does not even represent the amount extracted. It is of course impossible to say what relation exists between the amount extracted and the amount present, except that the amount present is much the greater, perhaps even several times as great. This same body has been obtained by extraction with sodium carbonate instead of hydrate, though the extraction is not so profitable.

PROPERTIES OF THE ISOLATED COMPOUND.

The properties of this compound, some of which have already been noted, are:

Slight solubility in cold water, more ready solubility in hot, ready solubility in alcohol and ether. It crystallizes in radiating clusters or concretions and when pure in very small plates or leaflets, which are generally arranged in starlike groups, as shown in the photomicrograph in Plate II, figure 1. The melting point is 98° to 99° C.

Calcium and barium salts are readily formed by decomposition of the corresponding carbonates. These are little soluble in cold water, but quite readily so in hot water. The barium salt crystallizes in somewhat characteristic circular concretions of radiating structure, as shown in the photomicrograph in Plate II, figure 2. The silver salt is amorphous and quite insoluble in water.

IDENTIFICATION AS DIHYDROXYSTEARIC ACID.

All the properties of this compound agree with those of dihydroxystearic acid prepared from elaidic acid. Elementary analyses of the preparation from the soil gave the following figures:

	No. 1 found.	No. 2 found.	Calcu- lated for $C_{18}H_{36}O_4$.
Carbon.....	67.5	68.0	68.3
Hydrogen.....	12.2	11.7	11.4
Oxygen.....	20.3	20.3	20.3

Analysis of the silver salt gave the following figures:

	Found..	Calculated for $C_{18}H_{36}AgO_4$.
Silver.....	25.77	25.53

The melting point of the soil compound remained unchanged when this was mixed with dihydroxystearic acid prepared from elaidic acid.

These figures, together with the correspondence noted between the properties of the body obtained from the soil and those of dihydroxystearic acid from elaidic acid, are sufficient to establish the identity of the soil compound as dihydroxystearic acid.

DIHYDROXYSTEARIC ACID AND RELATED COMPOUNDS.

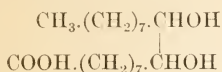
There are four isomeric dihydroxystearic acids known, three being laboratory products. The one found in the soil is that obtained in the laboratory by the oxidation of elaidic acid and described by Saytzeff.^a This acid can easily be prepared, starting with oleic acid. If oleic acid be treated for a few minutes at a low temperature with nitrous oxide generated in any convenient way, say by the action of nitric acid on arsenic trioxide, it is changed to the isomeric elaidic acid. This, after washing with water and recrystallizing from ether or alcohol, melts at 45° to 47° C. If this elaidic acid be dissolved in a dilute solution of potassium hydrate and treated with potassium permanganate, it is oxidized, dihydroxystearic acid being one of the products. The yield obtained in this way is low, the dihydroxystearic acid first formed being easily subject to further oxidation, pelargonic and azelaic, and finally oxalic acids being formed. Edmed^b in studying the oxidation products of oleic and elaidic acids obtained from the latter by this treatment with alkaline permanganate, dihydroxystearic acid, 33 per cent; pelargonic acid, 13 to 14 per cent; azelaic acid, 26 per cent; and oxalic acid, 15 to 20 per cent. The dihydroxystearic acid formed from elaidic acid is much more easily oxidized than the isomeric acid obtained by oxidation of oleic acid, and this has been

^a Jour. prakt. Chem., **33**, 300 (1886); **50**, 76 (1894).

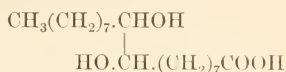
^b Jour. Chem. Soc., **73**, 627 (1898).

explained by the juxtaposition of the hydroxyl groups in the former, as shown in the structural formulas:

Dihydroxystearic acid, m. p. 99°,
from elaidic acid.



Dihydroxystearic acid, m. p. 134°,
from oleic acid.



The known dihydroxystearic acids other than that obtained by the oxidation of elaidic acid and found in soils are that obtained by the oxidation of oleic acid already mentioned, melting at 134° C.; one obtained from isoleic acid melting at 79° C., and a natural product found in castor oil melting at 140° to 141° C., and described by Juillard ^a and Meyer. ^b Besides the differences in melting points noted, these acids show quite important differences in their behavior toward solvents, but all agree in being little soluble in cold water. They are all monobasic acids, forming well-defined salts.

PRESENCE OF OTHER BODIES.

In describing the method used in isolating dihydroxystearic acid from the soil, mention was made of a resinous body which was present in the ether extract and which was separated from the dihydroxystearic acid after evaporation of the ether by filtration of the hot solution. This resinous material, which at the temperature of boiling water is a brown oil, becomes at room temperature a thick viscous mass. It is not soluble in water or dilute acids. In dilute alkalis it forms a slimy emulsion and dissolves readily in alcohol and ether.

So far no crystalline body has been obtained from this material, nor has it been possible to make any crystalline derivatives. No characteristic color or other reactions have been noted, and it is not known whether it is a simple body or a mixture. Elementary analysis gave the following figures: Carbon, 63.84 per cent; hydrogen, 9.65 per cent.

It is very insoluble in water and when wholly free from dihydroxystearic acid it does not impart any harmful properties to distilled water, using wheat seedlings.

POSSIBLE ORIGIN OF DIHYDROXYSTEARIC ACID.

Concerning the possible origin of dihydroxystearic acid in the soil, it might not be amiss to state briefly some of the results obtained, although no definite conclusion can as yet be drawn. Among the soils containing this compound was the Takoma soil, from which picoline carboxylic acid had also been isolated. This soil was found to contain dihydroxystearic acid in almost as large amount as that

^a Bul. Soc. Chim., **13**, 238 (1895).

^b Arch. Pharm., **235**, 184 (1897).

from Tennessee. This Takoma soil seems to be an extremely good medium for the growth of fungi, and it was noted that the rootlets of the oak trees on this ground were infested with mold. This growth was at times so abundant that in digging up the small roots and shaking free of soil, the attached mold mycelia furnished as much material as the roots themselves. A quantity of these roots with mold attached was washed free of adhering soil and treated just as the soil had been in the isolation of dihydroxystearic acid. There was obtained in this way a small quantity of crystalline material having the appearance and properties of the dihydroxystearic acid isolated from the soil. The crystalline form, melting point, solubility, formation, and appearance of barium salt all corresponded with those of the body isolated from the soil, and there is little or no doubt of their identity. Similar oak roots from another locality where they were not infested with mold were treated in the same way, but no indication whatever was obtained of the presence of dihydroxystearic acid or any other crystalline body by this method. It would seem to be a fair conclusion from this that mold can form dihydroxystearic acid from material associated with oak roots, and that in this case, at least, the dihydroxystearic acid found in the soil may have been formed in this way. As to what material furnishes the foundation for this formation of dihydroxystearic acid by fungi, whether it be root excretions or root tissue or cells, it is possible at present to theorize only, and such discussion does not come within the scope of this paper. It may, however, not be out of place to mention that the conditions existing in nature in this case are similar to those shown in the laboratory to lead to the formation of dihydroxystearic acid. Oleic acid as part of the complex molecules of fats and readily decomposed lecithins is present in all living parts of plants; nitrous acid, by which oleic acid is changed to elaidic acid, is constantly being formed in soils, either as a product of denitrification or as one of the stages in nitrification; and, finally, there are oxidizing agents—the air, enzymes in living roots, and micro-organisms.

SEPARATION OF OTHER ORGANIC COMPOUNDS FROM SOILS.

In the course of the examination of other soils for the presence of the compounds already described there have been obtained a number of bodies, some of which are undoubtedly mixtures, while others are evidently single, definite compounds. Two of these latter have been sufficiently studied to be mentioned here. The soil from which these bodies were obtained was from North Dakota, the Marshall clay, a black soil similar to the chernozem of Russia, containing 10.6 per cent organic matter and 0.51 per cent nitrogen.

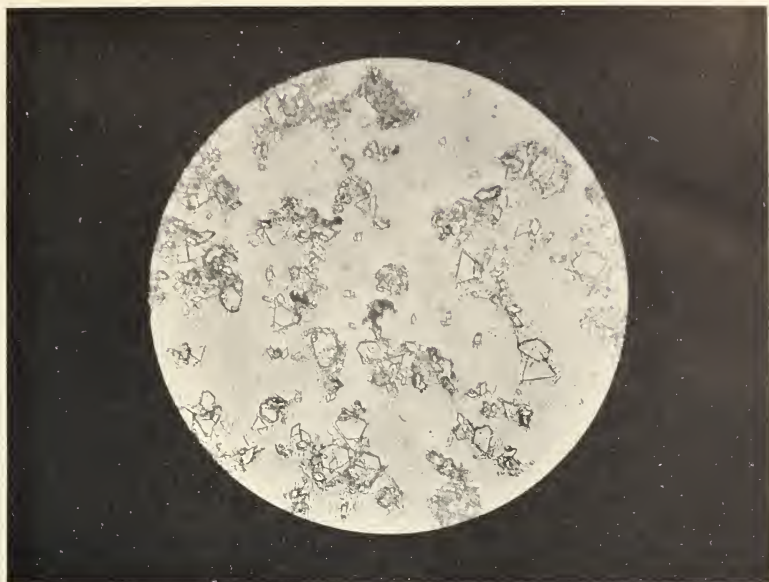


FIG. 1.—CRYSTALS OF AGROSTEROL OBTAINED FROM SOILS.



FIG. 2.—WHEAT SEEDLINGS GROWN IN EXTRACT OBTAINED IN THE METHOD FOR ISOLATING DIHYDROXYSTEARIC ACID FROM SOILS.

[1 and 2, undiluted extract; 3 and 4, one part extract, one part distilled water; 5 and 6, one part extract, nine parts distilled water.]

ISOLATION OF A WAXLIKE BODY; AGROCERIC ACID.

When this soil is treated with hot 95 per cent alcohol and filtered while hot, there is obtained a greenish-brown extract, from which on cooling a yellowish microcrystalline precipitate separates. If the soil be treated with successive portions of hot alcohol until the extract is no longer colored, the extracts combined, concentrated to a smaller volume, and allowed to cool, this precipitate can be obtained in considerable quantity. Two hundred and fifty parts per million of this substance have been obtained from this soil in this way, equivalent to 1,000 pounds per acre-foot.

The precipitate so obtained was washed with cold alcohol, redissolved in hot alcohol, and on cooling from this solution it crystallized fairly free of color. Thus obtained this crystalline body is evidently a mixture, since the melting point is quite variable as influenced by variations in the method, such as the concentration of the original alcoholic extract, extent of washing, etc. This variation in melting point extends from 70° to 90° C. If this mixture be thoroughly dried and washed with cold ether it is separated into two portions. The ether-soluble portion crystallizes on evaporation of the ether in minute leaflets which melt at 72° to 73° C. and solidify after melting at 67° C. It is little soluble in cold alcohol, readily in hot, the solution so obtained being acid to litmus. It separates from alcoholic solution on cooling in a powdery microcrystalline form.

On adding an alcoholic solution of lead acetate to the solution of this body in hot alcohol a precipitate containing lead is formed which after washing with cold alcohol and drying melts at 109° to 110° C.

The physical and chemical properties of this compound place it among the fatty acids found either free or combined in waxes both of vegetable and animal origin.

Elementary analysis gave the following figures:

	No. 1.	No. 2.
Carbon.....	73. 2	73. 40
Hydrogen.....	12. 2	12. 19
Oxygen.....	14. 6	14. 51

The only compound which corresponds with this percentage composition is that of a hypothetical acid, the lactone of which was found and described by Strucke^a in carnauba wax.

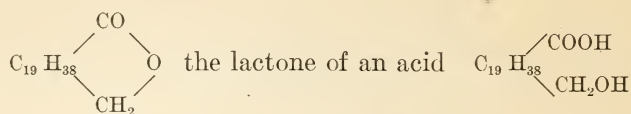
This acid, $C_{21}H_{42}O_3$, would have the composition—

	Per cent.
Carbon.....	73. 7
Hydrogen.....	12. 3
Oxygen.....	14. 0

and the figures obtained for the compound from the soil agree with these within the limits of experimental error.

^a Ann. Chem., **223**, 312 (1884).

Strucke isolated from carnauba wax and described a body to which he ascribed the formula



which is the one referred to above and which has not been described or isolated. Whether this compound isolated from the soil is the same as the acid corresponding to the lactone isolated from the carnauba wax can only be determined by further investigation. The compound from the soil, which will be designated as agroceric acid, is almost insoluble in water and does not impart any harmful properties to distilled water when wheat seedlings are grown, although it is not impossible that other plants may be affected differently.

PRESENCE OF OTHER WAXLIKE BODIES.

The portion of the original precipitate from the alcohol extract insoluble in cold ether is evidently a mixture of bodies. The melting point of this mixture varies from 100° to 110° C. and it contains from 3 to 7 per cent of ash, mostly alumina. Elementary analyses corrected for ash have given carbon, 74 to 77 per cent; hydrogen, 12 to 13 per cent. It is evident from its physical properties and composition that this body is a mixture of higher acids, salts of such acids, and probably higher alcohols such as make up natural waxes. This mixture, like the ether-soluble portion, is almost insoluble in water and does not form a solution harmful to wheat seedlings.

ISOLATION OF A COMPOUND OF THE CHOLESTEROL GROUP; AGROSTEROL.

When the hot alcoholic extract of the soil obtained as described above is concentrated, cooled, and separated from the precipitate of agroceric acid and other compounds the filtrate is a dark greenish-brown solution which on evaporation leaves a thick, somewhat resinous material. On washing this residue with cold ether there is left a yellowish waxlike body, the nature of which has not been investigated. The ether solution, which carries with it nearly all of the color of the original extract, on spontaneous evaporation of the ether leaves a resinous material similar to that obtained from the alcoholic solution. On treating this residue with cold absolute alcohol the coloring matter goes into solution and there is left a nearly white microcrystalline substance. This body melts at 237° C. and the melting point remains constant after recrystallizing from alcohol, ether, and chloroform. It crystallizes from ether in fine needles arranged in radiating clusters, from alcohol, if a little water is present,

in very thin plates of characteristic appearance, very similar to those of phytosterol. These crystals are shown in the photomicrograph in Plate III, figure 1. The compound is almost insoluble in water; difficultly soluble in cold alcohol, readily in hot, and readily soluble in ether and chloroform. It is unchanged on heating with alcoholic potash and can be obtained from the residue of the original alcoholic extract of the soil by treating this residue with alcoholic potash, evaporating to dryness, and extracting the dry residue with ether. The crystalline appearance and general properties of this body suggest a higher alcohol of the cholesterol group.

The color reactions ordinarily used to identify and distinguish members of this group when applied to this body gave the following results: Liebermann's "cholesterol reaction," which is the only one giving positive results with all the more commonly known members of the cholesterol group, gives the characteristic violet color with the compound obtained from the soil. This reaction consists in dissolving the substance in a small amount of chloroform, adding acetic anhydride, and then a drop of strong sulphuric acid, when a violet color is produced.

The other reactions ordinarily used in distinguishing compounds of this group gave no results with the soil compound. For instance, cholesterol when dissolved in chloroform and shaken with an equal volume of sulphuric acid (sp. gr. 1.76) gives a blood-red coloration in the chloroform layer. With the soil compound the coloration was light yellow. Again, when cholesterol is evaporated with strong hydrochloric acid containing 10 per cent of ferric chloride a violet-colored residue is left. With the soil compound no coloration is produced.

There have been quite a number of compounds described and named which seem to belong to this group of cyclic alcohols known as the cholesterol group and placed by Beilstein under the general formula $C_nH_{2n-8}O$. Most of these bodies are optically active and are found in fats and waxes, either free or combined. The combination is frequently one with fatty acids, the cholesterol in this case playing the same part that glycerol does in glycerides; cholesterol, however, and others of the group so far as known having only one alcohol or hydroxyl group.

Knowledge of the chemistry of this group is rather meager, cholesterol itself being the best known.

The compounds which have been described and placed in this group, together with their sources and some of their properties, are as follows: Cholesterol, $C_{26}H_{44}O + H_2O$, found in animal fats and in olive oil, melts at $148^\circ C$. Phytosterol, $C_{26}H_{44}O + H_2O$ "vegetable cholesterol," found in vegetable fats and waxes, melts at $132^\circ C$.

Isocholesterol, $C_{26}H_{44}O$, found in some animal fats, melts at $137^{\circ}C.$, does not react with chloroform and sulphuric acid. Sitosterol, $C_{27}H_{44}O + H_2O$, in plant fats, melts at $137.5^{\circ}C.$ Paraphytosterol, $C_{24}H_{40}O + H_2O$, in the seed coat of *Phaseolus vulgaris*, melting at 149° to $150^{\circ}C.$ Paracholesterol, $C_{26}H_{44}O + H_2O$, found in slime mold, melts at $134^{\circ}C.$ Caulosterol, found in etiolated yellow lupines, melts at 158° to $159^{\circ}C.$ Parasitosterol, $C_{27}H_{44}O$, found in the embryo of wheat, melts at $127.5^{\circ}C.$ Homocholesterol, $C_{28}H_{48}O$, found in Dalmatian insect powder (*Chrysanthemum cinerariæfolium*), melts at $183^{\circ}C.$ Ergosterol, $C_{26}H_{40}O + H_2O$, found in ergot, melts at $154^{\circ}C.$ Anthesterol, $C_{28}H_{48}O$, found in *Anthemis nobilis*, melts at 221° to $223^{\circ}C.$ Arnersterol, $C_{28}H_{46}O$, in *Arnica montana*, melts at 249° to $250^{\circ}C.$ Many of these bodies have as yet very doubtful standing as definite organic compounds, and much investigation will yet be necessary before the standing of these less-known bodies is assured, or the relationship between them is understood.

The composition of the compound obtained from the soil was found to agree very closely with the formula $C_{26}H_{44}O$. The analysis was made with material dried at $100^{\circ}C.$ and was as follows:

	Found.	Calculated for $C_{26}H_{44}O$.
Carbon.....	83.6	83.8
Hydrogen.....	11.5	11.8
Oxygen.....	4.9	4.4

In the compound isolated from the soil, as described above, we have, no doubt, a member of the cholesterol group. Its method of preparation, physical and chemical properties, so far as ascertained, crystalline appearance, and the cholesterol reaction are all evidence of this, and point to the existence in the soil of a compound of the cholesterol group. The cholesterol substance obtained from the soil does not correspond in melting point with any substance of this group so far described. For this compound, isolated from a soil, having the chemical properties and general appearance of substances of the cholesterol group, but differing in melting point from any of the members of this group so far described, the generic name *agrosterol* is suggested in harmony with the nomenclature of this group.

With regard to the origin of this compound in the soil, at least two possibilities present themselves: It will be seen that several members of the cholesterol group are so far as known found only in single species of plants. It may be that *agrosterol* is characteristic of some plant grown on this soil, and that on the decay of plants of this species it has survived the action of enzymes, fungi, and bacteria and remained in the soil as an unchanged plant residue. Since, however,

the presence of a substance of this group is shown by Liebermann's reaction above mentioned in several soils from widely separated localities with different native vegetation and cropping, it would seem that this suggestion has not much weight. To make this conclusive it would be necessary to show that the substances from different soils giving Liebermann's reaction are really identical, since the reaction is only a class reaction and not specific for agosterol¹ or any other member of the cholesterol group.

On the other hand, it is within the range of possibility that agosterol may be formed from some other substance through the agency of micro-organisms or chemical oxidation. The fact that Lifschütz^a has shown that a cholesterol substance can be formed by the oxidation of oleic acid emphasizes this possibility. The fact that paracholesterol mentioned above is found in slime molds further supports the suggestion that agosterol may be formed by micro-organisms.

CULTURE TESTS WITH THE ISOLATED COMPOUNDS.

In the study of soil fertility from the point of view stated in the first part of this paper, the first and immediate interest concerning any organic compound isolated from or proven present in a soil is with regard to the possible effect of such compound on plants. While it is probably true that every organic compound in the soil which is at all soluble is to some extent a factor influencing plant growth either favorably or unfavorably, and that before there can be any true science of agricultural chemistry the chemistry of the organic soil material, harmless as well as injurious, must be known, it is evident that at this stage the interest of the investigator, as well as of the practical agriculturist, centers around the injurious organic soil compounds. Once the identity of a soil compound is established, it can usually be prepared artificially in quantity sufficient for an extended study of its effects on plants, both alone and as modified by other soil compounds or added chemicals, fertilizers, etc., an investigation which can not but furnish much of scientific interest and practical value.

Anything like the complete knowledge of the organic matter of soils or even one soil can be arrived at only after long research, research in a field as yet almost unknown and one presenting many difficulties incident to ignorance of matter under investigation and inadequacy of available methods. Such research will follow as the natural result of the demonstration of the presence of inhibiting organic compounds in soils in the most positive and practical manner by isolating such bodies so that they can be seen, handled, and experimented with.

^a Zeitsch. physiol. Chem., 55, 1 (1908).

In determining the properties of bodies isolated from soils, wheat seedlings have been used as an indicator in the manner described in former bulletins.^a While wheat seedlings only have been used in these tests, it is not impossible that other plants may show inhibited growth where the wheat is unaffected, and vice versa, as was found to be the case in the experiment with plant succession already mentioned, in which harmful effects were noted when wheat followed wheat or oats, but wheat following corn or cowpeas was either unaffected or else benefited. In carrying out the tests, wheat seedlings three or four days old of uniform size were grown in solutions of the substances tested, control cultures of similar seedlings in pure distilled water being carried on at the same time. The transpiration and green weight of plants at the end of the period of growth, generally ten or twelve days, together with observations made from day to day on the appearance of the roots and tops furnished the data from which the conclusion as to inhibition was drawn.

EFFECT OF PICOLINE CARBOXYLIC ACID AND RELATED COMPOUNDS.

The method by which picoline carboxylic acid was isolated from soils was essentially the precipitation of the acid as either the lead or silver salt from a neutral solution of a portion of the soil organic matter. The amount obtained from the soil by this method was very small. For this reason, and more particularly to meet the objection that any observed injury might be due to minute traces of lead or silver incident to the method of preparation, the effect of this compound was tested with the artificially prepared body. The method of preparing this, which has already been stated, is in outline the treatment of pyruvic acid in alcoholic solution with dry ammonia gas, precipitation of uvitonic acid with sulphuric acid and splitting of this into picoline carboxylic acid and carbon dioxide on heating. The yield, however, is small.

The following figures give the results obtained with wheat seedlings grown for ten days in solutions of different strengths of artificial picoline carboxylic acid.

Effect of picoline carboxylic acid on wheat seedlings.

Solutions.	Relative transpiration.	Relative green weight.
Control in distilled water.....	100	100
Picoline carboxylic acid, 1 p. p. m.....	140	95
Same, 10 p. p. m.....	105	101
Same, 50 p. p. m.....	107	98
Same, 100 p. p. m.....	85	89
Same, 200 p. p. m.....	55	70

^a Buls. Nos. 23, 28, 36, 40, 47, Bureau of Soils, U. S. Dept. Agr.

It is seen from the figures above, substantiated by the results of other series, that this soil constituent is harmful to wheat seedlings and in low concentrations is a stimulant, an effect which is, however, characteristic of small doses of poisons when applied to plants. In the solutions containing 100 and 200 parts per million in addition to the decreased transpiration and green weight of plants the roots showed the abnormal appearance characteristic of the presence of an injurious compound, little growth with root tips first darkened, then club-shaped, or hooked. However, since this body does not show marked injurious properties in solutions containing 50 parts per million and so far as can be determined occurs in the bad soil extract from the soil examined in less amount, it does not seem likely to explain by its presence the full effect of the injurious properties of the extract from the soil in which it was found. While this effect, as will be shown, is undoubtedly due to the presence of the more harmful dihydroxystearic acid in this particular soil, nevertheless it was thought to be of interest to study in this regard several bodies closely related to picoline carboxylic acid.

Uvitic acid, from which picoline carboxylic acid is formed simply by heating to the melting point and which differs from it only by CO_2 , is so closely related that it was thought desirable to test its effect even though there was no evidence of its presence in the soil. The result of such a test with wheat seedlings grown for twelve days in solutions of different strengths of uvitonic acid is shown by the following figures:

Effect of uvitonic acid on wheat seedlings.

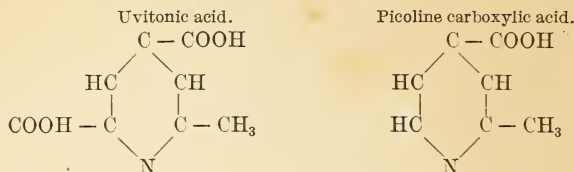
Solutions.	Relative transpiration.	Relative green weight.
Control in distilled water.....	100	100
Uvitic acid, 1 p. p. m.....	95	107
Same, 5 p. p. m.....	88	116
Same, 10 p. p. m.....	87	108
Same, 50 p. p. m.....	39	78
Same, 100 p. p. m.....	20	68
Same, 200 p. p. m.....	35	Dead.

The plants growing in concentrations of 200 parts per million were dead when the test was terminated, and the transpiration of this set is higher, owing to this death of the plants, as dying plants frequently show increased transpiration.

Uvitic acid, it is evident, is quite harmful to wheat seedlings, much more so than the nearly related picoline carboxylic acid. It is also reported to be strongly antiseptic, which is again an indication of highly injurious properties for higher plants.

The relative effect of these two compounds brings up the question of the relation of chemical constitution to inhibitory effect on plant

growth. Uvitic acid and picoline carboxylic acid are related as shown by the structural formulas



the former differing from the latter by CO_2 , which on heating splits off from the carboxyl. Uvitic acid, as shown, is a dicarboxylic acid, and the greater inhibition would seem to be associated with the presence of the second carboxyl group. However, all the carboxylic acids of pyridine and its homologues having one, two, and three carboxyls are weak acids. They form salts with bases in the usual way, but have also the power of forming salts with mineral acids by direct addition; for instance, the hydrochlorate, by the addition of hydrochloric acid. It is only in the higher members of this group, for instance, where all the H atoms are replaced by carboxyl, that the acid properties predominate and the power to form salts with mineral acids is lost. The whole question is one for future investigation, the data at present available furnishing little more than suggestions of possible explanations. It may be said, however, that the harmfulness of these compounds does not seem to be due to hydrogen ions.

Other related compounds, which may have a possible soil interest in this connection, have already been studied with the following results:^a

Pyridine ($\text{C}_5\text{H}_5\text{N}$) forms the nucleus upon which are built many of the alkaloids. It is obtained chiefly from coal tar, but may also be obtained from a number of alkaloids, such as nicotine, trigonelline, sparteine, and cinchonine, when these are highly heated, treated with alkalis, or distilled with zinc dust. Pyridine is a colorless liquid of penetrating odor and basic properties. Laurent^b found that a 1 per cent solution of pyridine was harmful to the fungi which he studied. It has been observed by Falkenburg^c that the vapor of pyridine and some of its homologues is poisonous to bacteria.

In the experiments with wheat seedlings it was found that although pyridine, in a concentration of 1,000 parts per million, did not kill wheat plants in nine days it was nevertheless very injurious, especially to the growth of the green parts of the plants. In a concentration as low as 50 parts per million the growth of the tops was inhibited and the leaf tips turned brown. In the lower concentrations there was no stimulation of growth.

^a Bul. No. 47, Bureau of Soils, U. S. Dept. Agr., 1907.

^b Ann. Soc. Belge Micr., 14, 29 (1890).

^c Czapek, Biochemie der Pflanzen, II, 926.



FIG. 1.—WHEAT SEEDLINGS SHOWING THE EFFECT OF DIHYDROXYSTEARIC ACID FROM SOILS.

[1, saturated solution of dihydroxystearic acid; 2, control in pure distilled water.]



FIG. 2.—WHEAT SEEDLINGS GROWN IN SOLUTIONS OF DIHYDROXYSTEARIC ACID FROM SOILS.

[1, solution of dihydroxystearic acid, 200 parts per million; 2, 100 parts; 3, 50 parts; 4, 20 parts; 5, control in pure distilled water.]



Picoline ($C_5H_4N.CH_3$) or methyl pyridine, was harmful to wheat plants, but only killed in the concentrations of 1,000 parts per million, and did not cause injury below 500 parts per million. The injury seemed to be manifested by the tops more than by the roots, thus resembling the action of pyridine.

Piperidine ($C_5H_{11}N$), or hexahydropyridine, is known to occur in nature, for example, in the pepper plant, and forms the nucleus of many alkaloids. Solutions of piperidine killed and injured at a lower concentration than either pyridine or picoline. A sample of piperidine that was neutralized with acetic acid proved to be more toxic than the strongly alkaline piperidine itself. Piperidine seems to injure the roots more severely than the tops.

Quinoline (C_9H_7N) has a genetic relationship with pyridine and forms the nucleus of many alkaloids found in plants belonging to the families Rubiaceæ and Loganiaceæ. Falkenburg^a found that quinoline was harmful to bacteria in a concentration of 2,000 parts per million. Quinoline killed wheat plants at a concentration of 500 parts per million. So low a concentration as 5 parts per million affected them injuriously in six days. None of the lower concentrations caused stimulation of growth.

While picoline carboxylic acid may not be an important factor in the infertility of the Takoma soil, the fact that it has been found in other soils in larger quantities, its moderately harmful effect, and its close relationship to other pyridine compounds still more harmful, places it among organic soil compounds to be considered and sought for in the general study of unproductive soils.

EFFECT OF DIHYDROXYSTEARIC ACID.

The dihydroxystearic acid isolated from the soil was readily obtained in quantity sufficient for cultural experiments, and as its method of preparation was such that it could easily be obtained free of any reagents which might have an injurious effect, the body obtained from the soil was used in testing the effect of this compound. As a preliminary test a saturated solution of dihydroxystearic acid, which was found to contain approximately 200 parts per million at room temperature, was used as a culture solution for the growth of wheat seedlings. The result is shown in Plate IV, figure 1. Ten days' growth of the seedlings in this solution gave a green weight of plants 52.5 as compared with 100 for the same number of seedlings in distilled water for the same time. The water transpired by the plants growing in dihydroxystearic acid

^a Czapek, *Biochemie der Pflanzen*, II, 926.

solution was 12.3 against 100 transpired by the plants growing in distilled water.

The harmful effect of this compound in not very high concentration being thus established, culture sets comprising several concentrations were then grown, the results of the ten days' growth being as follows:

Effect of dihydroxystearic acid on wheat seedlings.

Solutions.	Relative transpiration.	Relative green weight.
Dihydroxystearic acid 200 p. p. m.....	20	54
Same, 100 p. p. m.....	24	53
Same, 50 p. p. m.....	56	78
Same, 20 p. p. m.....	75	87
Control in pure distilled water.....	100	100

These results are illustrated in Plate IV, figure 2. The effect of this body, it will be noted, is as great in a concentration of 100 parts per million as in a concentration of 200 parts per million, which is approximately a saturated solution, and is marked even when the concentration is as low as 20 parts per million.

Artificial dihydroxystearic acid prepared as described from elaidic acid, as well as the isomeric dihydroxystearic acid formed on the oxidation of oleic acid, gave in concentration of 200 parts per million practically the same results, very little transpiration, green weight about half of that in the control solutions, and death of the plants in twelve or fifteen days. Other than this there has been no study of the effect of compounds related to the dihydroxystearic acid found in the soil.

Dihydroxystearic acid is, as may be inferred from its power to decompose carbonates, a much stronger acid than picoline carboxylic acid, and as represented by the formula $\text{CH}_3(\text{CH}_2)_7\text{CH} \cdot \text{OH}$ $\text{COOH}(\text{CH}_2)_7\text{CH} \cdot \text{OH}$ partakes also of an alcoholic nature. Whether the harmful effect of this compound is due to the combination of alcohol and acid characters, the so-called hydroxyacid structure, is of course not known. As has already been stated the whole question of the relation between effect on plant growth and composition and constitution is one which will be thoroughly understood only after long investigation. It may be well to note, however, in discussing the effect of a body of an acid constitution like dihydroxystearic acid that the data at present available is sufficient to prove that toxicity is not necessarily connected with the acid character or the presence of hydrogen ions. It is well known that an acid which is little dissociated in solution may be much more harmful than another which is dissociated to a much greater extent. For this as well as other reasons based on observations on the effect

of organic compounds many of those who have studied this question hold that the harmful effect of organic compounds is in many cases associated with the structure of the molecule rather than due entirely to dissociation products.

Preliminary investigations have shown that some of the combinations of dihydroxystearic acid with bases are as harmful as the acid itself, although much less soluble. It was found that cultures in the calcium and potassium salts of dihydroxystearic acid gave greater green weights and greater transpiration than cultures in distilled water, while the iron and alumina salts, although less soluble than the acid, gave a growth and transpiration almost as low as solutions of the acid itself. The iron and alumina salts, because the least soluble, are perhaps the ones most likely to be present in soils. It would seem, then, that the injurious effect of this body when found in the soil may not be confined to that produced slowly during its formation, but that it may be stored up in a little-soluble but very harmful form.

SUMMARY.

Previous investigations have indicated that soils may be infertile or "exhausted" because of the presence in them of organic compounds injurious to plants.

The present paper deals with the isolation and identification of some of the inhibitory or injurious compounds in such infertile soils. It is almost self-evident that where such bodies give rise to infertile soils there can be intelligent scientific treatment of such soils only when the nature and properties of these bodies are known. The isolation and identification of these bodies is the first step in the attainment of this knowledge.

In approaching this problem, which is an exceedingly complex one, it was found necessary to review briefly the knowledge of the origin of the organic matter in the soil. This review shows that the soil organic matter arises through the decay of organic compounds of vegetable and animal origin; that among these compounds are many of well-known composition and constitution which get into the soil and may even persist for a time, and that many compounds which are formed from these in the process of decay are likewise well established as definite chemical compounds.

When the effect of these compounds which are known to get into the soil or to be formed in the soil is considered, it is found that many of them have been shown to be harmful to plants, some even when in very small amounts. On the other hand, it is not yet possible to trace any general relation between the harmful properties and the chemical composition or constitution, and consequently it is not possible to determine the effect of any compound on plants except by experiment.

In addition to known products of vegetable and animal origin and their products of decay, it is possible that still other compounds are produced and exist in the soil about which there is no knowledge, not even of a speculative kind. In view of these facts, it was found necessary to consider briefly the present knowledge of soil organic matter in general. It was found that although the organic matter in soils is an important ingredient, amounting in most cases to many tons per acre, almost nothing is known of the chemical nature of this material.

The difficulties, then, in attempting to isolate harmful organic compounds from infertile soils are due to ignorance of what compounds are harmful to plants and what are not, and also to lack of knowledge of the chemical nature of the organic matter of the soil and consequent lack of methods for dealing with such material.

The first crystalline organic compound isolated from a soil was a nitrogenous body, picoline carboxylic acid, which was obtained by a process which had its starting point in a solution of a portion of the organic matter in sodium hydrate solution. This body is a definite crystalline compound with very faint acid properties and has also the power of acting as a base toward strong acids. Along with the isolation of this compound there were obtained indications of the presence in the same soil of other bodies, some of which are related to it.

From the same and another soil by a modification of the same method another organic compound was obtained. This compound, dihydroxystearic acid, is nonnitrogenous and is in no way related to the nitrogenous body first isolated. Dihydroxystearic acid, like picoline carboxylic acid, is a well-defined crystalline chemical compound heretofore known only as a laboratory product.

The properties of these two organic compounds were tested by the effect of their solutions on wheat seedlings. Picoline carboxylic acid was found to be harmful in concentrations of 100 parts per million, and like many poisons had a stimulating effect when present in small quantities. Dihydroxystearic acid was injurious in all concentrations tested and ultimately caused death of the plants in concentrations of 100 parts per million.

Two other crystalline organic compounds previously undescribed in the literature were isolated from a soil. One of these, which has been named agroceric acid, is a member of the waxy acids; the other, which has been named agrosterol, belongs to the cholesterol group of bodies. Neither of these substances appear to be harmful to wheat and are of interest in the study of soil fertility chiefly with regard to their possible connection with the generation or destruction of injurious or beneficial compounds, although it is not impossible that they may be themselves harmful to plants other than that tested.

Apart from the purely scientific interest attached to the isolation from soil of any definite organic compound, the work here stated furnishes a simple tangible proof of the presence of injurious organic compounds in soils and should lay the foundation of the rational study and remedy of unfavorable conditions in such unproductive or so-called "exhausted" soils.

In the present bulletin there are described four organic compounds isolated from soils, all well-defined crystalline bodies, one of which is quite injurious, one slightly harmful but related to much more harmful compounds, and two that are not harmful so far as the wheat seedlings used in the tests are concerned, although, as pointed out above, other plants may be quite differently affected by these substances.

In addition to these, there have been isolated a number of other well-defined bodies, likewise crystalline. These various soil compounds are being studied and as far as possible their nature and properties will be determined and the results given in future publications.

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